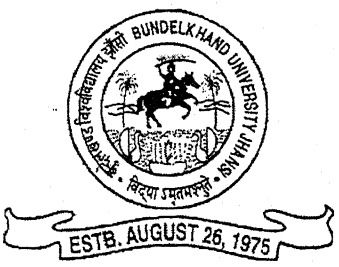
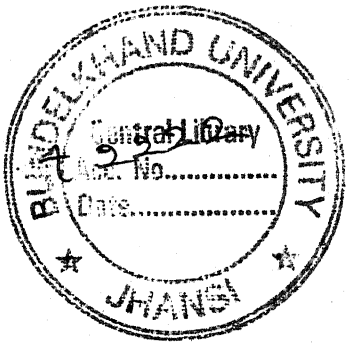
CHEMICAL STUDIES ON INDIGENOUS MEDICINAL PLANTS





THESIS

SUBMITTED TO THE

BUNDELKHAND UNIVERSITY, JHANSI

FOR

THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

Under the Supervision of

Dr. S. Q. Hasan

Ex - Principal and Head, Dept. of Chemistry
Pt. J. L. N. (P. G.) College,
Banda (U.P.)

By

Dinesh Kumar

Deptartment of Chemistry
Pt. J. L. N. (P. G.) College,
Banda (U.P.)

May 2005

Dedicated to

My Venerable

Grand Parents

Late Shri Bhikkhe

Smt. Brijrani

&

My Beloved

Parents

Shri Shivnath Singh

&

Late Smt. Rajabeti

Dr. S. Q. Hasan
M. Sc., Ph. D., F.I.C.C.
Ex - Principal and Head, Dept. of Chemistry
Pt. J. L. N. (P. G.) College, Banda (U.P.)

CERTIFICATE

This is to certify that the thesis entitled **"CHEMICAL STUDIES ON INDIGENOUS MEDICINAL PLANTS"** submitted in fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in chemistry is a record of bonafide research carried out by **Mr. Dinesh Kumar,** under my guidance and supervision at Pt. J. L. N. (P. G.) College, Banda (U.P.). This research work is suitable for submission to award the Ph. D. Degree. The work embodied in this thesis is original and such work has not been submitted elsewhere.

Jhansi : May 2005

(Dr. S. Q. Hasan)

ACKNOWLEDGEMENTS

At the very out set, I feel it my bounden duty to evince my heart-felt gratitude towards my learned supervisor, **Dr. S. Q. Hasan,** a teacher a 'Pole– Star' to all wandering and shipwrecked person like me, for his invaluable guidance, concrete and constructive suggestions which paved my thorny way towards the high-hill tops of success. It is his whole hearted support, concerted co-operation and very meticulous scientific attitude which enkindled in me an insatiable urge to work constantly to accomplish this up-hill task rather in a jiffy.

After the almighty, omniscient God the person who deserves the highest credit for lightning me towards my bright life is **Prof. Ramesh Chandra** Hon'ble Vice-Chancellor, Bundelkhand University, Jhansi. By whose blessing, I could complete my research work. Further, I am also indebted to him for his benign help and encouragement during the course of present study.

I gratefully remember Princiapl **Dr. N. L. Shukla** Pt. J. L. N. (P.G.) College, Banda, for wishfully providing me facilities necessary for the successful execution of my work, all faculty members and my senior research fellows of the department.

I would like to forward special thanks to **Dr. R. S. Dixit,** R. R. I. A. Jhansi, for providing some seed samples etc.

A bouquet of thanks to Directors **Prof. P. S. Visen** and **Prof. R. C. Rajak,** Bundelkhand University, Jhansi, for their encouragement and suitable help.

Authors bigest thanks go to **Dr. R. S. Kushwaha,** Dept. of Chemistry B.B.C. College, Jhansi, for his encouragement and constructive criticism during the research work.

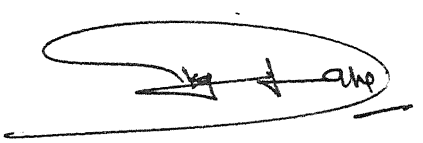
Deep down in the inner most recesses of my heart, I am much indebted to **Shri M. P. Singh**, Dept. of Information and Library Science, B.U. Jhansi, for full support and co-operation provide during my research assignments.

I would like to express my deepest gratitude and obligation to **Dr. Rambeer Singh** and **Dr. Sunil Babu,** for their utmost co-operation, considerate atitude and constant encouragement.

I want to express heartiest gratitude towards my elder brothers **Mr. Roop Singh, Devendra Kumar** including respected my brother in-laws **Mr. Parashuram Gautam, Mr. Hardash Gautam** and elder sisters **Smt. Rekha Gautam, Smt. Saroj Gautam** who were the first to lay down the foundation of education to me on which I have been able to construct this accumplishment of today. Their prayers and blessings have always been with me like the guiding stars showing me the right direction.

In fine, I would like to thank those persons also who have directly or indirectly helped me in achieving this task.

I fully appreciate the hard work carried out by **Mr. Ajay Agarwal** (4, Behind Pachkuiyan Mandir, Jhansi) for neat and accurate computer type setting and printing the manuscript efficiently in due time.



(Dinesh Kumar)

CONTENTS

General Introduction

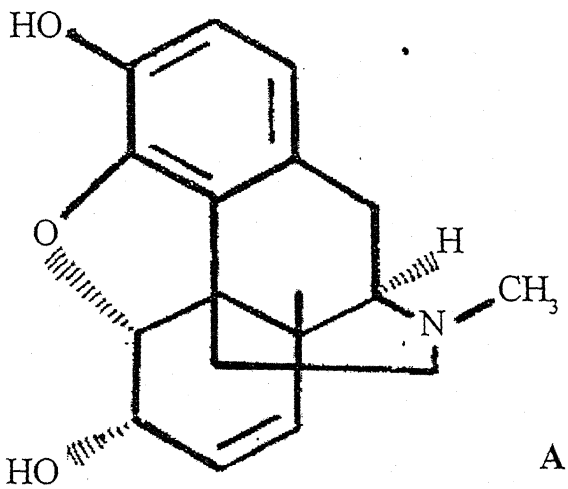
GENERAL INTRODUCTION

The close Relationship between man and plants Played an important role in the health care of human being since the dawn of civilization. This relationship led to the evolution of different systems of medicine like Chinese, Ayurvedic, Unani and Allopathic etc. Such system of medicine are based on the considerable amount of knowledge accumulated through application of individual of observations followed by the popularization through Medical Practitioners. These traditional / folk medicines in one form or the other, is wide spread throughout the world and are the part of the tradition of each country where it is handed down from generation to generation **[1,2]**.

Man as a knowledge seeker, gradually learnt the mysteries of active principles of Medicinal plants with continuous refinement in understanding their principles leading to the discovery of many bioactive substances. Recent studies revealed that not only tradition medicinal plants, but also many wild plants e.g. *Catharanthus roseus, Artemisia annua* and *taxus* spp. etc. earlierconsidered as medicinal herbs of great significance **[3-7]**.

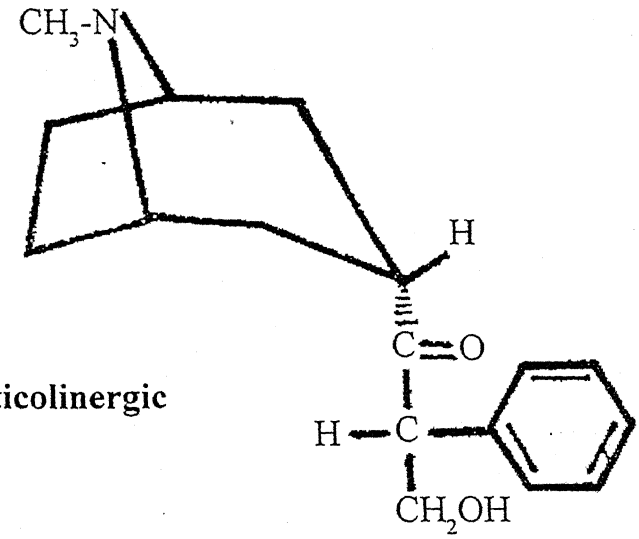
Indian materia medica shows that more than 80% of the drug mentioned there, are of plant origin, spicieses in Indian food are mainly used for their Medicinal Properties **[8]**. Not only India, but also in other countries people are relying upon plants for their medicinal function.

The plants are the good source of economically important Natural Products such as oils, resins, Tannins, Rubber and Pesticides drugs. Almost every pharmaceutical synthetic drug or preparation include a Natural Product Prototype e.g. Morphine in analgesics and atropine (dl-hyoscyamine) in anticolinergic agents etc. therefore, plants still remain as an untapped reservoir of potentially useful compounds not only as drugs but also as a starting material for synthetic analogues and as interesting tool that can be applied to a better understanding of biological processes. However, in spite of such a wide scope as drug or Precursor of drugs many plants are yet to be explored more systematically and exhaustively for their drug value.



Morphine

A Prototype for analgesics



A Prototype for anticolinergic

Atropine (dl-hyoscyamine)

Plants are unique chemists, they synthesize variety of chemicals during their routine life reactions in order to protect themselves from the herbivores viz. insects, grazers and against pathogens. These chemicals can be subdivided into two categories as primary metabolites viz. carbohydrates, proteins, vitamins, enzymes and lipids etc. which are invariably required for their existence and secondary metabolites e.g. alkaloids, terpenoids, flavonoids and esteroids. Many a times these secondary metabolites directly or indirectly effect. Human /Animal health on their ingestion either due to their therapeutic value or toxic nature. Most of the plants, which are harmful to man in large quantities, provide good medicinal products for various diseases on their use in small and regulated quantities. So it is obvious that these substances are of vital importance to us and it is the chemists who have to share the responsibility of exploring an identifying these secondary metabolites.

A good number of plants have been adopted in pharmacopoeia after systematic chemical and pharmacological investigations. However, far and large numerous are still waiting for inclusion to invigorate the indigenous system of medicine. In a country like ours, this type of research has a vast scope due to her diversified natural wealth specifically medicinal flora. A large number of plants have found place in the literature through the vast experience of saints, sages, and scholars and are reported in the ancient medical literatures **[9-15].** Majority of plants is still waiting for the investigations for assessment of claims made for them. Now it is up to the phytochemical researchers to explore this infinite treasure. Due to the benefits without side affects, even people from urban areas are also reverting to the traditional Ayurvedic system; this is a very encouraging sign for the phytochemical research.

Generally, it is observed that most of the bioactive phytochemicals are oxygenated in one form or the other. The fact can be authenticated by many examples, for instance artemisinin from *Artemisia annua* (an endoperoxide sesquiterpene and potent antimalarial drug effective against multidrug resistant *plasmodium falciparum*) **[3,4]**; *Taxol,* from *taxus* spp. (a diterpenoid anticancer drug) **[5,6]**; vineristine and vinblastine, from *Catharanthus roseus* (binary indole alkaloids, the anticancer drugs) **[7,16]** (fig No. 1.1)

There are Numerous oxygenated Natural Products having been divided into several classes based on their structure and biogenesis. The highly abundant, widely distributed and diverse group of Natural Products are the Terpenoids which con-

tribute distinctly in the field of Medicinal and aromatic plants. The Terpenoids, have been described to be uniquely important in the formation of vesicles and cell-membranes in organic evolution [17]. They also play other roles including insect development harmones, plant growth harmones, and mammalian sex harmones besides many important drugs viz. artemisinin and taxol etc. [3-6].

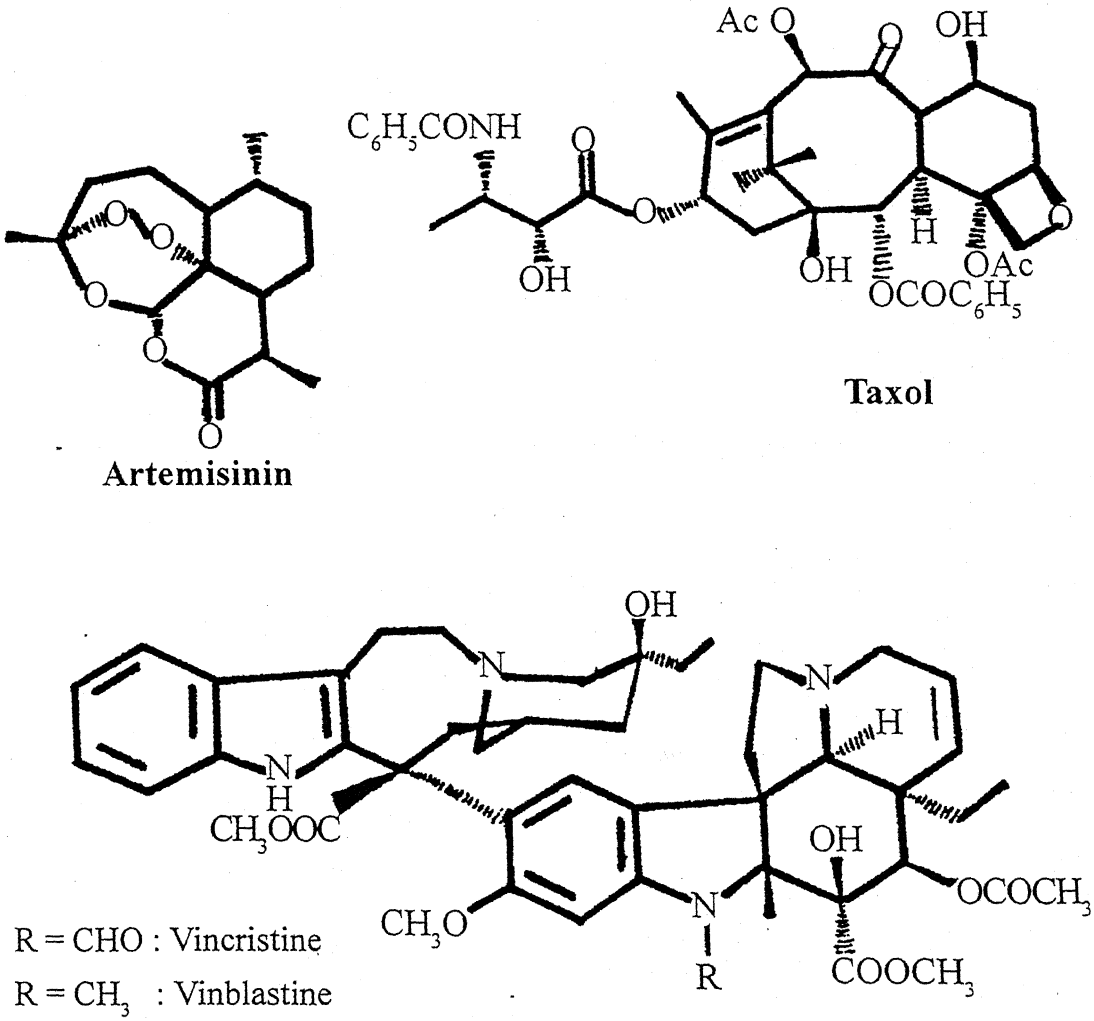


Fig 1.1, Some of the medicinally valuable oxygenated Natural Products.

All terpenoids, may be considered to be formed by the assembly of a whole number of five carbon units derived from 2- methyl butadiene. As early as 1887, Wallach conjectured that the Terpenoids are formed from Isoprene units. Ruzika (1953), after about three decades of dedication to the study of terpenoids, made following hypothesis in to a general rule **[18, 19].**

The precursors for the chief classes of terpenoids are the genesis of the reactions catalyzed by enzymes, from phosphoric esters of $(C\text{-}5)_n$ unit (s) via isopentenyl pyrophosphate **(IPP)** into a starter molecule, namely an allylic prenyl pyrophosphate. The first unit in the series being dimethyl allyl pyrophosphate **(DMAPP),** followed by geranyl pyrophosphate **(GPP)** (Precursor of C_{10} monoterpenoids), farnesyl pyrophosphate **(FPP)** (Precursor of C_{15} sesquiterpenoids), geranyl geranyl pyrophosphate **(GGPP)** (Precursor of C_{20} diterpenoids), geranyl farnesyl pyrophosphate **(GFPP)** (Precursor of C_{25} sesterpenoids). However, C_{30} triterpenoids arise from squalene which is the resultant of the head to head reductive coupling of two farnesyl pyrophosphate units ($2\times C_{15} = C_{30}$) **[20, 21,22].** In few cases the isoprene rule does not seem to be followed. For example, the irregular" monoterpenoids (e.g. pyrethric acid, santolinatriene) arise from the coupling of two DMAPP units, by a mechanism similar to the one which lead to triterpenoids **[23,24]** fig. [1.2 & 1.3]

Biogenetically, it seems that most simple among the terpenoids are mono and sesqui-terpenoids, because they arise from the coupling of two and three iso-

prene units, respectively **[18, 25, 26].** But owing to the variety of intramolecular cyclizations, oxidation and rearrangement reactions, they form very large number of structures. Essential oils, which can be obtained by distillation of a wide array of plants, contain only the volatile terpenoids, i.e. mostly mono-and sesqui-terpenoids.

Triterponoids, around four thousand compounds built upon over 40 different skeletons are C-30 compounds arising from the cyclization of 3S-2,3-epoxy-squalene or from the squalene itself **[20, 27, 28]**. Mostly, oxygenated at 3-position arising from the opening of the epoxide, the triterpenoids, present very high structural homogeneity. The major difference in configurations are linked to the conformation adopted by the squalene or its epoxide prior the cyclization. The cation resulting from this cyclization can subsequently undergo a series of 1,2-proton and methyl group shifts, which can be used to rotionalize the occurrence of the different tetra and pentacyclic skeleta, characteristic of each group **[29]**. fig. (1.4).

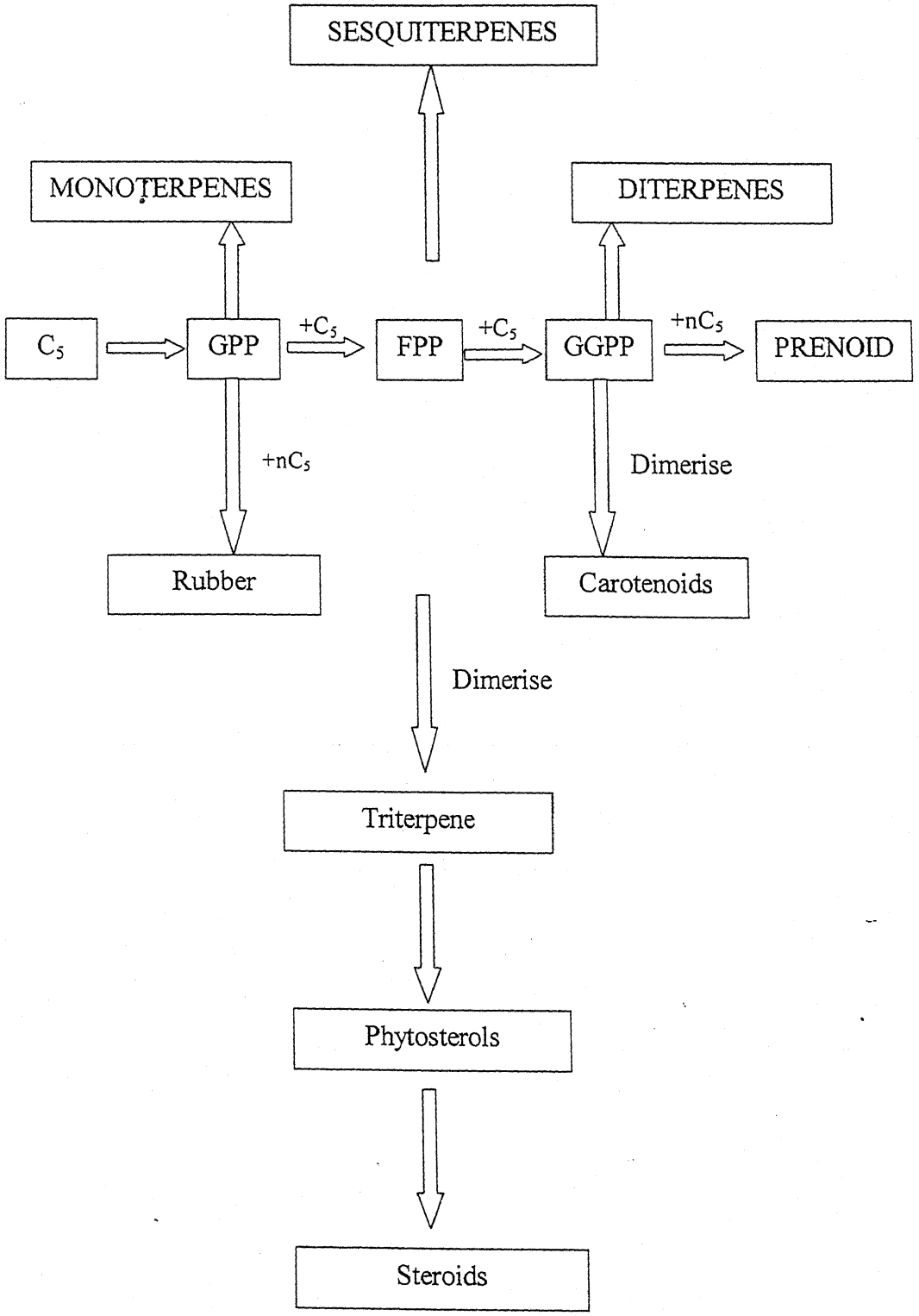


Fig. - 1.2 The general biosynthetic pathway of terpenoids

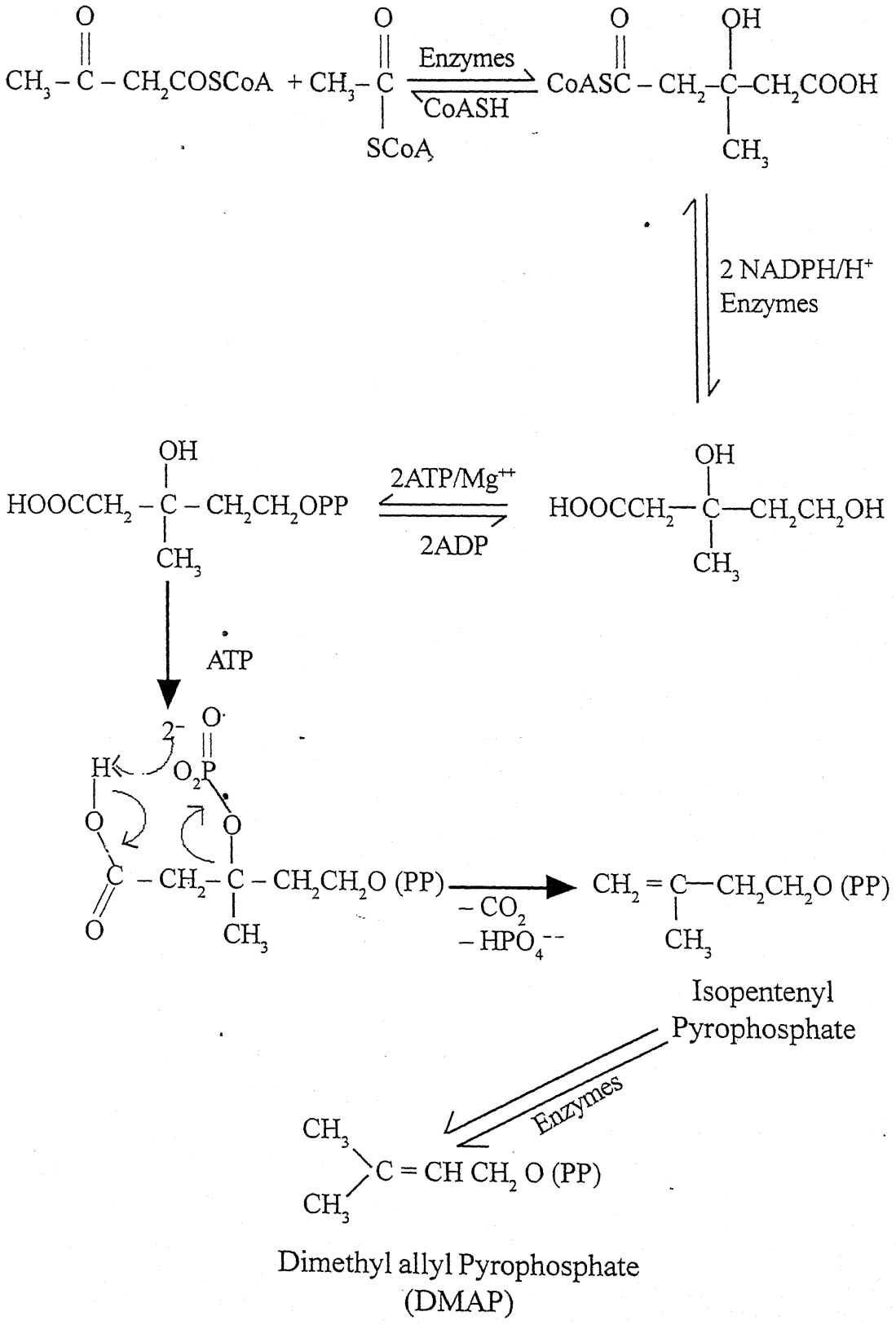


Fig No. - 1.3 Genesis of DMAP, a precursor of terpenoids

Flavonoids are wide spread plant natural products and are consumed in measurable amount by human. The estimated daily dietary is 1gm/day in the united state **[30]**, while evaluation of most flavonoids indicated that these natural product are safe for human consumption, recent studies on flavonoids indicated the diversity of pharmacological and physiological effects of these plant natural products. Various flavonoids have been demonstrating to have anticarcinogenic activity and antimutagenic activity **[31-36]**. Flavonoids such as quercetin were found to be mutagenic in Ame's test **[37]**. Flavonoids also influence the incidence of breast cancer by acting as antiestrogens **[38]** or as aromatase inhibitors **[39]**.

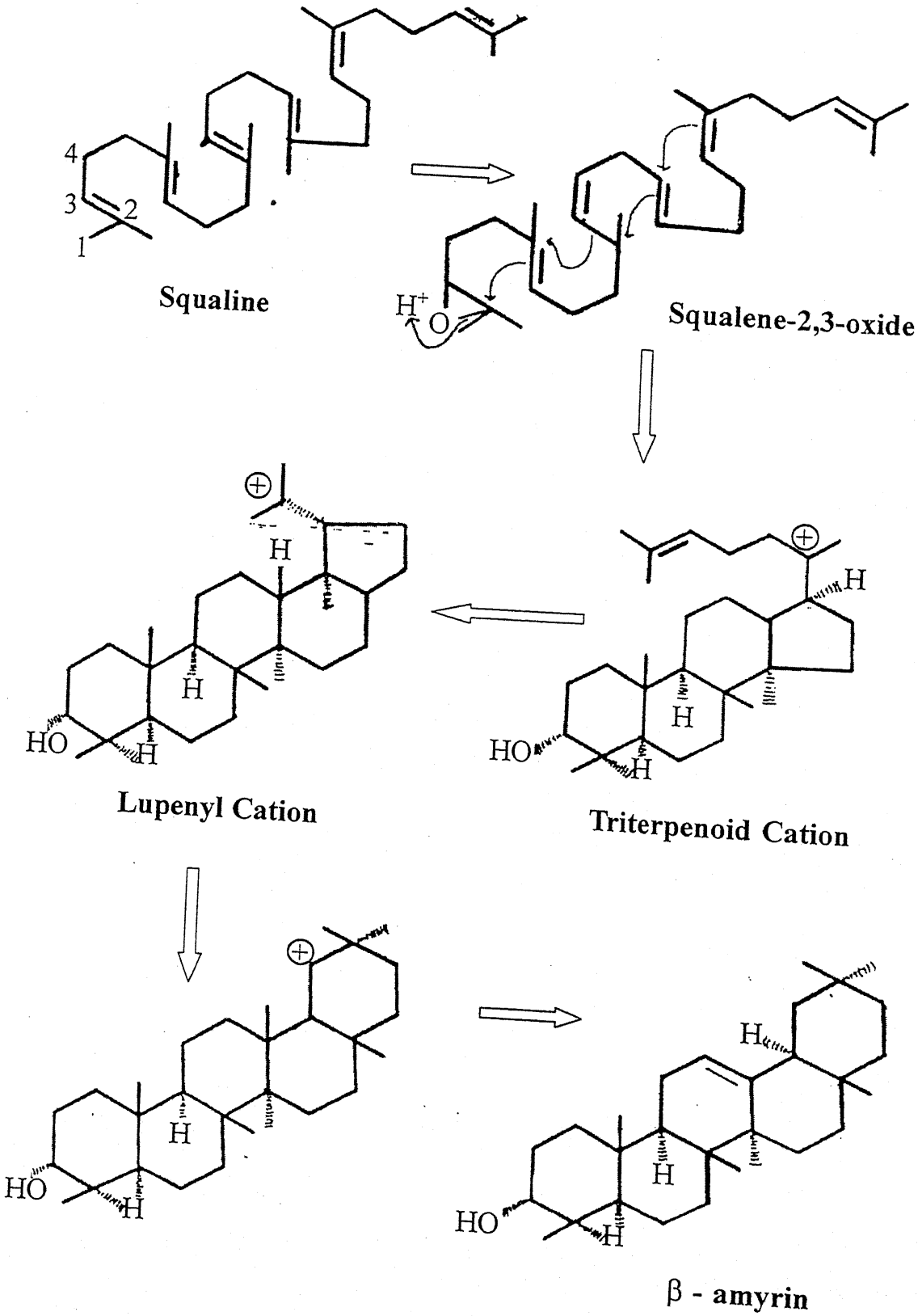


Fig. - 1.4 Biogenetic pathway of triterpenoids

LIPIDS :-

As far as the study of fats and their fatty acids is concerned fats attained much less attention as compared to the more favoured carbohydrates, alkaloids and terpenoids etc. Recently the new class of lipids are under much investigation, out of these the phospholipids and sphingolipids etc. are the old one where as a comparatively newer class of lipids is "cyanolipids". Though the development and progress of organic chemistry is made in different phases out of which the chemistry of fats and their fatty acids has allowed it to reach near perfection. As we know that almost all the types of lipids contain the long chain fatty acids, the study of long chain fatty acids is very important part of the fat analysis. Some fatty acid derivatives find applications in pharmaceuticals.

India has once again emerged as one of the major importer of edible oils. As a result of liberal policy, edible oil imports have jumped from 1.75 million tonnes in 1996 - 97 to a high of 4.4 million tonnes last season. In the first five months of current years, the import figures stood at 1.57 million tonnes compared to 1.15 million tonnes during the same period last year. The provisional figures for March 2000 stand at 307, 724 tonnes against 158, 740 tonnes in March 1999.The flooding of market with cheap imported edible oils would make domestic oil seed production and processing unviable.

On the other hand the government of India has decided to continue the oil seed production programme with a total outlay of 760 crores during the remaining

two years of the 9th plan (1997-2002) and hope that a result, the area under major oilseed crops could increase to 30 million hactares by the end of the 9th plan as against 26.4 million hactares at the end of the 8th paln period. The government also expects oil seed production to reach 30 million tonnes at the end of the current plan period as against 24.38 million tonnes during the previous plan period. The average productivity levels during the same period is expected to increase to 1057 kg/Ha from 926 kg/Ha and the average oil availability to 9 million tonnes from 7.5 million tonnes.

The industry on its part has also not geared itself to face the challenges of globalization. Technology upgradation has hardly taken place in this sector. This together with poor quality standards have contributed to poor economic viability of it's operation. It is time to take a fresh look at the options available to make this industry, which is second only to the petroleum sector in terms of turn over, rejuve-nate itself.

Thus the road map for the Indian oil seed and vegatable oil industry in the new millennium is anything but clear. The potholes and the road blocks have to be identified and a strategy chalked out to eliminate them. This convention attempt to take a relook at the oil seed and oil sector and suggest ways to improve the health of this vital industry, experts from the industry, government agencies and R& D institutions could discussed at length and make appropriate recommendations.

The cyanolipids which is a new class of plant lipids are found only in the

seed oil of Sapindaceae plants and probably play an important role in the biochemistry of these plants. Although the occurrence of cyanolipid materials has been suspected for many years **[40-43]** due primarily to the cyanogenic property of Kusum seed oil, only recently **[44-63]** have their structures been determined and the existence of this class of lipids acknowledged **[64-65]**.

The unifying structural feature of these cyanolipids is that they are all based on the same branched, five-carbon nitrile skeleton, although the double bond position and the number and location of hydroxyl groups are not the same. Two of the four known cyanolipids do not fit in the "diol lipid" category **[66]** because they are monoesters, and cyanolipids as a group can not be labelled "cyanogenic lipids" because two of them do not liberate HCN. The unique structures of this new class of lipids enable them to undergo reactions that are distinctive from any other lipid class.

However some earlier reports on unidentified "cyanogenic glycosides" may, infact, deal with cyanolipids. This facet remains unexplored. In addition, it is easy to imagine how investigators who were not specially searching for cyanolipids would not have detected them; in year past, cyanolipids have been repeatedly overlooked in seed oils containing them in large amounts.

Being a relatively new development, the cyanolipid field does not possess a large body of literature as do other lipid subdivisions. In order to build a firm basis for further discussion, a brief synopsis of cyanogenesis is provided, particularly as

it relates to results of Kusum oil research obtained prior to the actual structure proof of cyanolipids. Cyanolipids appear to occur only in sapindaceae plants; therefore, a sampling of pre-cyanolipid literature describing research on some of the more important plants of this family (In addition to Kusum) has also been made.

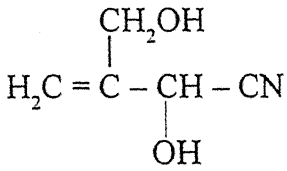
Because these lipids undergo distinctive and interesting reactions, detailed informations and some experimental procedures were deemed beneficial and have been included. Results of NMR analysis are throughly explored because this technique has proven most definitive in sorting out the various cyanolipid structures. Biosynthetic studies on cyanolipids are just beginning to yield returns.

CYANOLIPIDS :

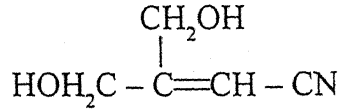
Up to the time that the true nature of cyanolipids was discovered. *Schleichera trijuga* (Kusum) was the sapindaceous plant that had been most throughly investigated, specially it's seed lipids Kusum seed oil is used variously as a medicinal oil, a hair dressing, an edible oil and a raw material for soap production. The cyanogenic property of Kusum oil has long been recognized and documented **[40-41]** and numerous reports describing its fatty acid and triglyceride composition have appeared **[67-70].** Oleic acid was usually reported **[71-73]** to be the major constituent of this oil, ranging from 40-62%. It was also generally agreed that Kusum oil had little or no linoleic or linolenic acids, but that relatively large amounts (20-25)% of arachidic acid were observable by methods available to the investigators.

More recently, GLC has provided ample confirmation of these earlier conclusions by analysis of mixed methyl esters prepared from Kusum oils **[69-70]**. In addition, Dhar **[68]** actually isolated and characterized arachidic acid from saponified Kusum oil. However, Basu **[74]** recently published a somewhat sketchy report showing a GLC analysis of Kusum oil methyl esters that disagrees with all other published data. He reports 50% linoleic acid, 22% oleic acid and only 1% of C_{20} acids. The reason for wide variation from generally accepted values is unclear.

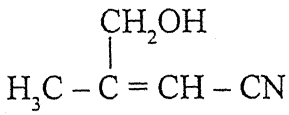
In early 1970's the structures of cyanolipids have been determined which are related to five-carbon nitrile moiety differing in the position of double bond and the number and location of hydroxyl groups (a-d).



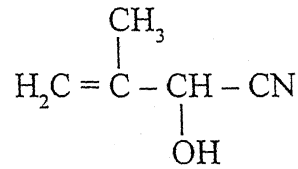
(a)



(b)



(c)



(d)

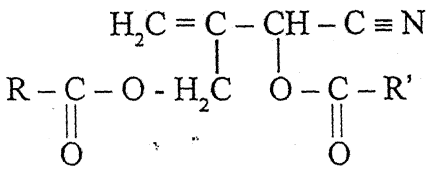
Progress in cyanolipid identification began with reports **[42, 43]** on Kusum oil by workers in India, even though their data and conclusions were misleading. At about the same time Mikolajczak et al., in a series of publications **[46,50,75]** described the detection, isolation and structure proof of four types of cyanolipids having different but closely related structures.

In *Schleichera trijuga* the exact location of the cyanogenic moiety in the oil or it's exact nature was not reported. The compound has been suspected to be in the form of a cyanogenic glucoside or an amide **[76]**. Later, Kundu et al. **[42]** reinvestigated the same seed oil to ascertain the location and nature of the cyanogenic compound by applying chemical methods, chromatography and infared spectrocopy. Observations indicated the cyanogenic compounds to be a part of glyceride molecule in which one of the hydroxyl group of latter is bonded to the cyanogenic compound through an ether linkage. Chromatographic behaviour of the

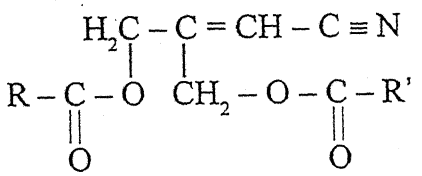
isolated cyanogenic compounds further indicated that atleast two glyceride molecules are involved. These glycerides are predominantly esterified with saturated fatty acids. At the same time Kasbekar and Bringi **[43]** working on the same seed oil found with the help of TLC that the oil is composed of appoximately 37% of glyceride, the rest being non-glycerol esters of fatty acid.

Later studies **[44, 46-51, 54, 75]** have shown that the cyanogenic material is non-glycerol ester composed of one or two ordinary fatty acid moieties (predominatly C_{20}) esterified with an unsaturated isoprenoid hydroxy or dihydroxynitrile.

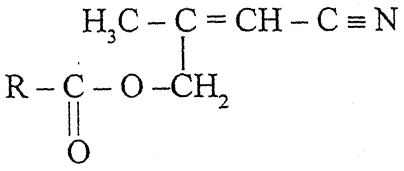
Four types of cyonolipids (I-IV), present individually or in pairs, have been indentified in the seed lipids which are cyanogenetic non-glycerol esters and are derivatives of five-carbon mono or dihydroxynitrile moiety esterified with long chain fatty acids (I-IV). Out of these, one class of compound is a mixture of diesters containing two fatty acids moieties esterified with I-cyano-2-hydroxymethylprop-2-ene-1-ol (I) and l-cyano-2-hydroxymethylprop-l-ene-3-ol (II). The other class of cyanolipids comprise mono-ester of l-cyano-2-methylprop-l-ene-3-ol (III) and l-cyano-2-methylprop-2-ene-l-ol (IV).



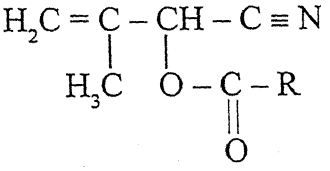
(I)



(II)



(III)



(IV)

Each cyanolipid fraction is a mixture in which the constituents differ only in the attached fatty acids; because this mixture was difficult to separate and appeared to be based on a single aglycone, it was treated as a single entity during the course of investigation.

A number of sapindaceous plants other than Kusum have been quite throughly investigated [72] because of economic interest, and some possess interesting properties or constituents. Among these plants are *Blighia sapid*s (akee) fruit, a major food of Jamaica [77] which contains toxic polypeptides when immature [78]. *Sapindus* seed, which produces saponins [79 - 81] in large quantities, is used in soap manufacture; nothing particularly unusual was noted about its oil [82 - 84], however.

Paullinia cupana seed is ground and used extensively in Brazil to brew a

coffee or tea-like drink called guarana **[85]**. Edible fruits and seed oils **[72 - 86]**, (which contain large amount of C_{20} acids) are derived from *Nephelium lappaceum* and *N. mutabile*.

Seeds of *Koelreuteria paniculata* (an ornamental) reportedly are insecticidal to mosquito larvae, and extracts of *Sapindus saponaria* and *Paullinia fuscescens* plants exhibit toxicity to leaf feeding insects.

Seed oil of *Ungnadia speciosa* was reported to contain a cyanogenic glycoside **[87]**, and to produce certain unpleasant effects, likely caused by HCN, when the oil was eaten as a salad oil. Apparently this glycoside has not been characterized.

Dihydrosterculic acid, was shown to be a conspicuous compounent (17%) of glycerides of *Euphoria longana* seed oil **[88]**. This is the only known report of cyclopropanoid acids occurring in sapindaceous plants, but they may also be found in *Litchi chinensis* seed oils **[89]**.

One particularly noticeable feature of Sapindaceae seed oil is that a majority of them comprise large amounts (up to 72% in *Paullinia meliaefolia*) [90] of C_{20} fatty acids; **[46, 90 - 92]**, these may be saturated, unsaturated (primarily monoenoic) or a mixture of both.

More recently the proesence of cyanolipids has been shown in *Sapindus saponaria* **[61]**, *Sapindus trifoliatus* **[62]** and *Lepisanthes tetraphylla* **[63]** of Sapindaceae family by Hasan et al.

A curious feature of these cyanolipid containing seed oils is their high percentage of C_{20} acids and the preferential incorporation of these acids into cyanolipids rather than into the accompanying triglycerides.

Cyanolipids have thus far been found only in seed oils of Sapindaceae plants, hawever, not all sapindaceous seed oils contain cyanolipids **[46 - 50, 75]**. Even though, oil from plants of other families have been screened for cyanolipids, but non have been detected. *Schleichera trijuga* seed oil yields the largest total amounts of cyanolipid, but seed oils of some *Koelreuteria*, *Cardiospermum* and *Paullinia* **[40,46,50]**, species are close behind. Of course, seeds from different accessions may display differing amounts of cyanolipids depending on such variables as growing location, seed maturity and storage conditions. Various seed oil of sapindaceous plants have been analyzed for cyanolipid contents by Seigler **[51]**, but no new cyanolipids were found. A comparative study of cyanolipids revealed that the cyanolipid (I) is by far the most abundant and usually occurs either or with a minor amount of cyanolipid (II), while cyanolipid, (III) appears, for the most part, in combination with cyanolipid (II). Whereas no oil, examined to date has contained all four cyanolipids. Cyanolipid (IV) has been detected in only one seed oil, that of *Ungnadia speciosa* **[46, 51]**. A review of the literature reveals that members of a particular genus usually produce the same cyanolipid (s).

The search for cyanolipids in plants other than Sapindaceae has been extended by Seigler and Kawahara **[53]** to include members of four families viz.

Meliosmaceae, Melianthaceae, Hippocastanaceae and Styphyleaceae which are closely allied botanically to the Sapindaceae, but none of seed oils examined gave any indication of the presence of cyanolipids.

Only a meager amount of research has thus far been reported concerning how these strange cyanolipids are produced in plants. Significantly, the hydroxynitrile moiety in all four cyanolipids has an isoprenoid skeleton this permits numerous possibilities for its biosynthesis. Since other natural cyano compounds often seem to be derived from amino acids or their procursors, it should be noted that decarboxylation of L-leucine would give the requisite saturated carbon and nitrogen skeleton for these nitriles.

REFERENCES

[1] N. R. Fransworth, in "Natural products and drug development", Alfred Benzon Symposium 20, P. Krogsgaard-Larsen, S. B. Christensen and K. H. Munksgaard, eds; Copenhagen, P. 17 (1984).

[2] N. Anand and S. Nityanand, in "Natural products and drug development", Alfred Benzone Symposium 20, P. Krogsgaard-Larsen, S. B. Christensen and K. H. Munksgaard, eds; Copenhagen, P. 78 (1984).

[3] L. Jing - Ming, N. Mu - Yun, F. Yu - Fen, T. You - You, W. Zhao - Hoe Yulin and C. Wei - Shan, Acta. Chim. Sinica, 37. 129 (1979).

[4] Anonymous China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials. J. Trad. Chin. Med., 2, 17 (1972) .

[5] M.C. Wani. H. L. Taylor, M. E. Wall, P. Coggon and A.T. Mc. Phail, J. Am. Chem. Soc., 93, 2325 (1971).

[6] M. Suffness, Am. Rep. Med. Chem., 28, 305 (1993).

[7] W. I. Taylor, N. R. Farnsworth, eds. the Catharanthus Alkaloids : Botany, Chemistry, Pharmacology and Clinical uses, Marcel Dekker, New York (1975).

[8] J. F. Dastur, "Medicinal plant of India and Pakistan". By D. B. Taraporevals Bombay P. 2 (1970).

[9] R. N. Chopra, K. L. Handa and L. D. Kapoor, Indigenous Drugs of India,

2nd ed. U. N. Dhar and Sons Calcutta (1958).

[10] R. N. Chopra, S. L. Nayar and I. C. Chopra, "Glossary of Indian medicinal plants", C.S.I.R., New Delhi (1933).

[11] K. R. Kirtikar and B. D. Basu, "Indian medicinal plants", Indian Press, Allahabad, Vol. I, IV. (1933).

[12] K. M. Nadkarni, "Indian materia medica ", Popular Book Dept. Bombay (1954).

[13] P.C. Ray, "History of the chemistry in Ancient and medieval India - Incorporating the history of hindu - chemistry", Indian Chemical Society, Calcutta (1956).

[14] Wealth of India - Raw materials, C.S.I.R., New Delhi (1969).

[15] H. R. Zimmer, "Hindu medicine", The Jhone Hopkins Press, Baltimore (1948).

[16] G. A. Cordell, in "Progress in phytochemistry", L. Reinhold, J. B. Harborn and T. Swain, Vol. 5, Pergamon Press, Oxford P. 273 (1978) .

[17] G. Ourisson and Y. Nakatami, Chemistry and Biology, 1, 11 (1994).

[18] L. Ruzika, Experientia, 9, 357 (1953).

[19] L. Ruzika, J. Pure and Appl. Chem., 6, 493 (1963).

[20] K. Bloch, Science, 150, 19 (1965).

[21] A. Eschenmoser, L. Ruzika, O. Jeger and D. Arigoni, Helv. Chim., Acta 38, 1890 (1955).

[22] G. Stork and A. W. Burghstahlu, J. Am. Chem. Soc., 77, 5068 (1955).

[23] D. V. Banthorpe and B. V. Charlwood, in "secondary plant products encyclopedia of plant physiology", Vol. 8, E. A. Bell and B. V. Charlwood, eds., Springer - Verlag, Heidelberg, New York, P. 185 (1980).

[24] J. Shaw, T. Hoble and W. Epstein, J. Chem. Soc., Chem. Commun., 590 (1975).

[25] G. Rucker, Angew. Chem., Int. Edn., 12, 793 (1973).

[26] W. Parker, J. S. Roberts and R. Ramage, Qt. Rev. Chem. Soc., 21, 331 (1967).

[27] J. D.Connolly and R. A. Hill, in "Methods in plant biochemistry", Vol. 7, Terpenoids, eds, B. V. Charlwood and D. V. Banthorpe, eds., Academic Press, London, P. 331 (1991).

[28] J. R. Sabine, Cholesterol, Marcel Dekker, New York (1977).

[29] A. Eschenmoser, L. Ruzika, O. Jeger and D. Argoni, Helv, Chim. Acta, 38, 1890 (1955).

[30] J. Kuhnau, "World rev. nitr. diet", 24, 117 (1976).

[31] R. K. Bhattacharya and P. F. Firozi, "Cancer lett.", 39, 85 (1988).

[32] A. R. Francis, T. K. Shetty and R. K. Bhattacharya, "Mutat, res.", 222, 393 (1989).

[33] M. E. Wall, M. C. Wani, G. Manikumar, P. Abraham , H. Taylor, T. J. Hughes, J. Warner and M. R. Givney , "J. Nat. prod.", 51, 1084 (1988).

[34] D. F. Birt, B. Walker, M. G. Tibbels and E. Bresnick, "Carcinogenesis" 7, 959 (1986).

[35] R. Kato, T. Nakadate, S. Yamamoto and T. Sugimura "Carcinogenesis" 4, 1301 (1983).

[36] T. Konoshima, E. Okamoto and M. Kozuka "J. nat. prod"., 51, 1266 (1988).

[37] B. N. Ames, "Science", 221, 1256 (1983).

[38] B. Y. Yang and N. R. Adams, "J. endocrinal", 85, 291 (1980).

[39] J. T. Kellis and L. E. Vickery, "Science", 225, 1032 (1984).

[40] L. Rosenthaler. Schweiz Apoth. Ztg. *58*, 17 (1920); Chem. Abstr., 14, 556 (1920).

[41] N. N. Sengupta, J. Soc. Chem. Ind., *39*, 88 (1920); Chem. Abstr, 14. 2011(1920).

[42] M. K. Kundu and C. Bandyopadhyay, J. Am. Oil Chem. Soc., 46 23 (1969).

[43] M. G. Kasbekar and N. V. Bringi, Ibid., 46, 183 (1969).

[44] K. L. Mikolajczak, D. S. Seigler, C. R. Smith, Jr. and I. A. Wolff, R. B. Bates, Lipids, 4, 617 (1969).

[45] K. L. Mikolajczak, C. R. Smith, Jr. and L. W. Tjarks, Lipids, 5, 672 (1970).

[46] K. L. Mikolajczak, C. R. Smith, Jr. and L. W. Tjarks, Lipids, 5, 812 (1920).

[47] K. L. Mikolajczak, C. R. Smith, Jr. and L. W. Tjarks, Biochim. Biophys. Acta, 210, 306 (1970).

[48] D. S. Seigler, K. L. Mikolajczak, C. R. Smith, Jr. and I. A. Wolff, Lipids, 4, 147 (1970).

[49] D. S. Seigler, F. Seaman and T. J. Mabry, Phytochemistry, 10, 485 (1971).

[50] K. L. Mikolajczak, C. R. Smith, Jr., Lipids, 6, 349 (1971).

[51] D. S. Seigler, K. L. Mikolajczak, C. R. Smith, Jr. and L. W. Tjarks, Phytochemistry, 13, 841 (1974).

[52] D. S. Seigler, C. S. Butterfield, Phytochemistry, 15, 842 (1976).

[53] D. S. Seigler and W. Kawahara, Biochem. Systematics and Ecology, 4, 263 (1976).

[54] G. Gowarikumar, V. V. S. Mani and G. Lakshminarayana, Phytochem., 15, 1566 (1976).

[55] D. S. Seigler and D. Kennard, Phytochem., 16, 1826 (1977).

[56] K. L. Mikolajczak, Prog. Chem. Fats other lipids, 15, 97 (1977).

[57] D. Charles, Q. G. Ali and S. M. Osman, Chemistry and Industry, 2 (1977).

[58] I. Ahmad, A. A. Ansari and S. M. Osman, Chemistry and Industry, 19 (1978).

[59] M. R. K. Sherwani, S.Q. Hasan, I. Ahmad, F. Ahmad and S. M. Osman, Chemistry and Industy, 4 (1979).

[60] R. Ahmad, I. Ahmad and S. M. Osman, Chemistry and Industry, 4 (1985).

[61] S. Q. Hasan, Y. A. Roomi and Ms. Chitra Nigam, J. Oil Tech. Assn. India, 26, 77 (1994).

[62] S. Q. Hasan, Y. A. Roomi, J. Oil Tech. Assn. India, 28, 23 (1996).

[63] Y. A. Roomi, V. K. Srivastava and S. Q. Hasan, J. Oil Tech. Assn. India, 30, 65 (1998).

[64] C. Hitchcock, In Recent Advances in the Chemistry and Biochemistry of Plant Lipids p. 4 (T. Galliard and E. I. Mercer, eds.) Academic Press, London (1975).

[65] C. Hitchcock and B. W. Nichols, Plant Lipid Biochemistry, p. 50, Academic Press, London (1971).

[66] L. D. Bergelson in Progress in the Chemistry of Fats and Other Lipids, Vol. 10, Part 3, p. 241 (R. T. Holman, ed.) Pergamon. Oxford (1969).

[67] M. H. Coleman, J. Am. Oil Chem. Soc., 42, 751(1965).

[68] D. N. Dhar, Fette Seifen Anstrichm., 70, 942 (1968).

[69] S. N. Koley, M. D. Kundu and A. N. Saha, Indian Oil Soap J., 30, 321 (1965); Chem. Abstr. 64, 14430 h (1966).

[70] B. Sreenivasan. J. Am. Oil Chem. Soc., 45, 259 (1968).

[71] D. R. Dhingra. T. P. Hilditch and J. R. Vickery, J. Soc. Chem. Ind., 48, 281 T (1929); Chem., Abstr., 23, 5601(1929).

[72] E. W. Eckey, Vegetable Fats and Oils, p. 624, Reinhold, New York (1954).

[73] T. P. Hilditch, The Chemical Constitution of Natural Fats, 3rd Ed., p. 231. John Wiley, New York (1965).

[74] A. K. Basu, Curr. Sci., 43, 410 (1974).

[75] Idem, Lipids, 5, 672 (1970).

[76] R. L. Datta, T. Basu and P. K. Ghosh, Indian J. Soap, 16, 71 (1950).

[77] P. R. Ashurst, J. Sci. Res. Counc. Jam., 2, 4 (1971); Chem. Abstr., 75, 85128 u (1971).

[78] C. H. Hassall, K. Reyle and P. Feng, Nature, 173, 356 (1954).

[79] W. A. Jacobs, J. Biol. Chem., 64, 379 (1925).

[80] P. C. Maiti, S. Roy and A. Roy, Experientia, 24, 1091 (1968).

[81] L. R. Row, C. Ruchmini, Indian J. Chem., 4, 36 (1966); Chem. Abstr., 64, 15948e (1966).

[82] O. C. Dermer and L. T. Crews, J. Am. Chem. Soc., 61, 2697 (1939).

[83] A. Sengupta, S. P. basu and S. Saha, Lipids, 10, 33 (1975).

[84] K. Singh, P. C. Bansal, A. Jyoti and I. Sengupta, Indian J. Chem., 9, 21 (1974); Chem. Abstr., 82, 5548 n (1975).

[85] R. H. Cheney, Econ. Bot., 1, 243 (1947).

[86] G. C. Manalac and M. F. Collantes, Philippine J. Sci., 93, 67 (1964); Chem. Abstr., 62, 9364e (1965).

[87] E. Cheel and A. R. Penfold, J. Soc. Chem. Ind., 38, 74 T (1919).

[88] R. Kleiman, F. R. Earle and I. A. Wolff, Lipids, 4, 317 (1969).

[89] R. Kleiman, Personal Communication.

[90] T. P. Hilditch and W. J. Stainsby, J. Soc. Chem. Ind., 53, 197 T (1934).

[91] M.J. Chisholm-and C.Y. Hopkins, Can. J. Chem., 36, 1537 (1958).

[92] C.Y. Hopkins and R. Swingle, Lipids, 2, 258 (1967).

CHAPTER - 1

Chemical Investigation, Isolation and Characterization of a New Triterpene from *Salvia coccinea*

INTRODUCTION

Salvia Coccinea belogs to family Lamiaceae/Labiatae (mint family). Common names of *Salvia Coccinea* are Scarlet sage, Texas sage and Salvia. Scarlet sage is a subshrub perennial in warmer climates and an annual where winter temperatures stay below freezing for more than a few hours at a time. Scarlet sage is a good bedding plant and useful in borders where brilliant color is desired. This is especially desirable in natural area gardens where it attracts butterflies and hummingbirds and maintains itsef year after year without dominating or becoming invasive.

EXPERIMENTAL

All m.ps. are uncorrected. Whole plant of *S. Coccinea* was supplied by the M/s united chemical and Allied products, Kolkata, India.

Air dried defatted powdered whole plant (1.5 kg) of *S. Coccinea* was extracted with benzene in a soxhlet apparatus for 56 hours. The extract was concentrated under reduced pressure and then subjected to CC on silica gel (60-120 mesh, 200g). The chloroform eluent afforded the compound **1** (1.5g), m.p. 150-151^0, ^{1}H NMR (90 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$) spectral data are discussed in the text, EIMS, m/z 484 (M^+, 20%), 469 (M–CH_3, 1.5%), 425 (M-$COOCH_3$, 25%), 262 (retro-Diels-Alder fragmentation around ring C, 100%, base peak), 221

(M–262–H, 35%), 203 (221–H_2O, or 262–$COOCH_3$, 75%), 133 (60%).

Acetylation of Triterpene 1 : The triterpene (**1**, 0.05 g) was dissolved in a solution of 5 ml acetic anhydride and 2 ml of pryridine. The reaction was allowed to stand for four days at room temperature. Then the solution was poured into cold water, extracted with ether and dried when an acetate of **1** (0.06 g), m.p. 140^0 was obtained.

Hydrolysis of triterpene 1 : The triterpene (**1**, 0.5 g) was refluxed with 20% ethanolic KOH (10 ml) for 8 hours, then the solvent removed H_2O added and the mixture filtered.

The residue on repeated CC over silica gel (60-120 mesh, 50g) furnished virgatic acid (**3**, 0.07g), $C_{30}H_{46}O_4$ $[M]^+$ m/z 470, m.p. 225-26^0, ν_{max} (KBr) 3400, 1720, 1695 cm^{-1}, ^1H NMR (90 MHz, $CDCl_3$) δ 0.70 (3H, s), 0.85 (6H, s), 0.90 (6H, s), 0.98 (3H, s), 1.20 (3H, s) for seven tertiary methyls, δ 2.30 (2H, m) ascribed to keto methylene protons, δ 3.55 (1H, t) assignable to carbinol methine proton, and δ 5.40 (1H, m) for a vinylic Proton.

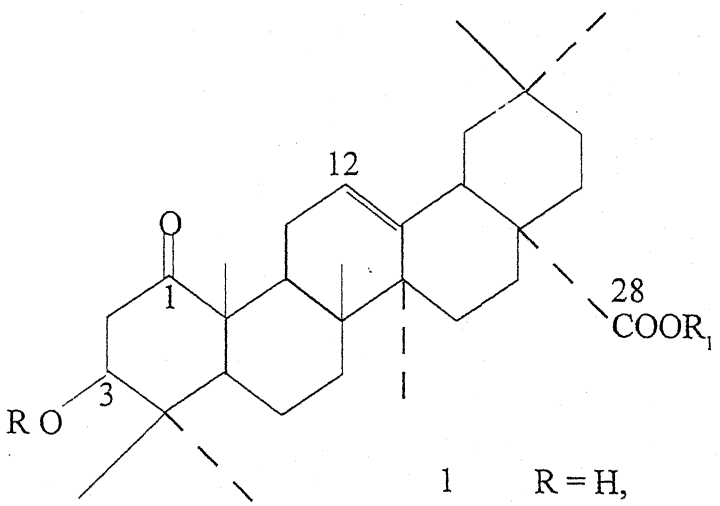
RESULTS AND DISCUSSION

We report herein the isolation and characterization of a New Triterpene from the benzene extract of this plant.

The Concentrated benzene axtract of the defatted whole plant (aerial parts and roots) of *S. Coccinea* on chromatographic analysis over silica gel (60 - 120 mesh) using solvents with increasing polarity furnished a white solid, $C_{31}H_{48}O_4$ (M^+, m/z 484), crystallized from acetone, m. p. 150 - 151^0 in chloroform eluent. It responded positively to Liebermann - Burchard test for Pentacyclic triterpene. IR spectrum showed bands at ν_{max} (KBr) 3440 (OH), 1735 (ester carbonyl), 1720 (six memberd Cyclic Carbonyl) and 1660 cm^{-1} (unsaturation).^{1}H NMR Spectrum displayed signals for seven tertiary methyls at δ 0.75 (3H, s) 0.80 (3H, s), 0.85 (3H, s), 0.93 (3H, s) 0.98 (6H, s) and 1.25 (3H, s), two protons multiplet at δ 2.30 assignable to $-CH_2-C=0$, a three protan singlet at δ 3.75 for carbomethoxy function, one proton multiplet at δ 3.52 (b., half band width 15 Hz) for a carbinol methine proton and, a vinylic proton at 5.25 (1H, m). The compound readily formed an acetate, $C_{33}H_{50}O_5$ (M^+, m/z 526) **(2)** m.p. 140^0, on treatment with acetic anhydride in pridine and a 2, 4 - dinitrophenylhydrazone derivative, m.p. 170-72^0. The mass spectrum of the parent triterpene is typical of Δ^{12}- oleanene sketleton [1] and from the mass values of the significant peaks it is evident that the carbomethoxy group is in the D/E ring portions while keto and hydroxyl functions are in A/B ring portion.

On hydrolysis with ethanolic KOH, the parent triterpene formed an acid, $C_{30}H_{46}O_4$, m.p. 225-226°, ν_{max} (KBr) 3400, 1720, 1695 cm^{-1} which was found to be identical with 3β-hydroxy-1-oxo-olean-12-en-28-oic acid (virgatic acid) **(3)** **[2]** by direct comparison of physical and spectral data with authentic sample (m.m.p., co-IR and co-TLC. this settles the locations of keto and hydroxyl functions respectively at C–1 and C–3 positions and carbomethoxy group at C–17 position in the parent compound. These led us to formulate this triterpene as methyl 3β–hydroxy–1–oxo–olean–12–en–28–carboxylate **(1)** which appears to be the first report of occurrence of this triterpene from natural source.

The structure of the compound **1** also received support from its ^{13}C NMR data (Table 1) which are comparable to those compounds having similar skeleton **[3]**.



1	R = H,	$R_1 = CH_3$
2	R = OAc,	$R_1 = CH_3$
3	R = H,	$R_1 = H$

Table 1. ^{13}C NMR chemical shift of compound 1

C	δc	C	δc
1	218.2	17	48.4
2	36.7	18	42.6
3	75.6	19	46.6
4	47.3	20	31.5
5	55.4	21	34.8
6	19.8	22	33.7
7	34.1	23	26.8
8	40.8	24	21.1
9	50.1	25	17.5
10	37.0	26	18.1
11	23.9	27	26.3
12	123.6	28	178.2
13	144.1	29	32.6
14	42.7	30	23.8
15	28.7	OMe	51.7
16	24.9		

CHAPTER - 2

Chemical Investigation of Seeds of *Pongamia pinnata* L.

INTRODUCTION

Pongamia, a genus of only one spp. (Indo-malayan) : *Pongamia Pinnata* L. (Syn. *Pongamia globara* vent.), belongs to the family leguminosae and the sub-family papilionacea. It is generally grown as avenue tree. It is used medicinally in China, Indo-China, the phillippine Islands, Australia [4]. Seeds of the plant have very high medicinal value as these are useful in inflammations, pectoral diseases, chronic fevers and anaemia [5] and we have undertaken the reinvestigation of the seeds.

EXPERIMENTAL

Seeds (2 kg) of *P. Pinnata* were collected from Chitrakoot area of Bundelkhand region the plant seeds were identified in department of Botany Pt. J.L.N. (P.G.) college, Banda, U.P.

The seeds were separated from the seed coat and then extracted first with hexane and then with methanol. The column chromatography of methanolic extract afforded five compounds (**1-5**). Elution with benzene - petroleum ether (1 : 3) afforded 2'-methoxy-β-hydroxyl (2", 3" : 4', 3') furanochalcone (**1**, 310 mg), ethyl acetate - benzene (1:19) karanjin (**2**, 30 mg), lanceolatin B (**3**, 30 mg), pongaglabrone (**4**, 40 mg) and 3', 4' - dimethoxy (2", 3" : 7, 8) furanoflavone (**5**, 20 mg). Melting points were determined on Ganson electrical melting points apparatus. IR spectra

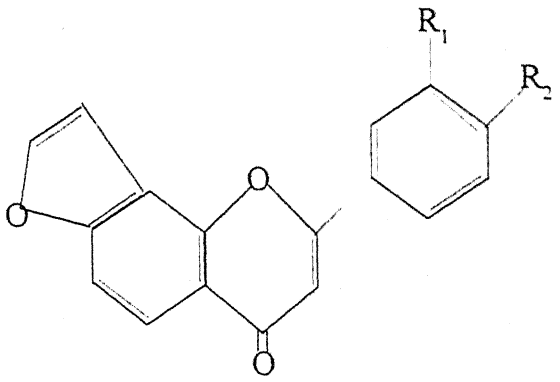
were recorded on Hitachi 570 infrared spectrophotometer using KBr. ^{1}H NMR spectra were recorded on Brucker AC-300 F MHz NMR spectrophotometer using TMS as internal standard. Chemical shifts are given in δ (ppm) and $CDCl_3$, was used as solvent for taking NMR spectra. Mass spectra were recorded on VG-70S 11- 250J GC-MS-DS mass spectrophotometer.

Compound **5** was obtained on elution with ethyl acetate - benzene (1:19). Yield 20 mg, m.p. 171-172^0 (Found : C, 70.79; H. 4.32. $C_{19}H_{14}O_5$ required : C, 70.80; H. 4.34%); UV λ_{max} 267, 301 nm; IR ν_{max} 515, 647, 771, 856, 926, 1073, 1156, 1370, 1482, 1629, 2362, 2838 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) 7.93 (1H, d, J 7.5 Hz, H-5), 7.90 (1H, dd, J 7.5 and 2.5 Hz, H-6'), 7.75 (1H, d, J 2.0 Hz, H-5"), 7.49 (3H, m, H-6, H-2' and H-5'), 7.16 (1H, d, J 2.0 Hz, H-4"), 6.77 (1H, s, H-3), 4.09 (6H, s, 2 × OMe), MS m/z (rel. int.) 325 (M^+ +3, 7.2), 307 (1.8), 292 (100), 264 (3.6), 165 (5.0), 162 (35.5), 160 (16.3), 131 (2.6), 116 (2.1), 90 (1.7).

RESULTS AND DISCUSSION

The column chromatography of the methanolic extract of the seeds revealed the presence of five compounds **(1-5)**. Out of these, 2'-methoxy-β-hydroxyl (2", 3" : 4', 3') furanochalcone **[6] (1)**, Karanjin **[7] (2)**, lanceolatin B **[8] (3)** and pongaglabrone **[9] (4)** are known compounds.

Compound **5** was obtained as colourless crystalline solid. Colour reaction with Mg/HCl indicated the compound to be a flavone. Its UV spectrum (λmax 267 and 301 nm) supported the compound to be a furanoflavone **[10].** The IR spectrum showed the presence of a carbonyl group (1629 cm^{-1}). The MS and elemental analysis suggested $C_{19}H_{14}O_5$ to be the molecular formula for the compound. Its ^1H NMR spectrum showed the presence of two doublets (J 2.0, Hz) at δ 7.75 and 7.16 for H-5" and H-4' and furano protons. A singlet at δ 6.77 was observed for H-3 proton and a multiplet was observed at δ 7.49 for H-6, H-2 and H-5' protons. A double doublet (J 7.5 and 2.5 Hz) at δ 7.90, was observed for H-6' proton. A doublet (J 7.5 Hz) at δ 7.93 was assignable to H-5. A singlet was observed for six protons at δ 4.09, which showed the presence of two methoxy groups. Based upon this data, compound **5** was assigned the structure 3', 4'-dimethexy (2", 3" : 7, 8) furanoflavone **(5).** The RDA fission in the MS fragmentation pattern suggested the presence of methoxy groups in the B-ring by showing peaks at **160** (A-ring fragment) and 162 (B-ring fragment).



5 $R_1 = R_2 = OMe$

CHAPTER - 3

Chemical Investigation of

Athyrium solenopteris (Kunze)

INTRODUCTION

Athyrium solenopteris (kunze) T. Moore, an Athyroid fern is growing on fully shaded stream banks, forest floor and fully exposed marshy place. It is commonly available in India. Plant materials were identified from Ayurveda reasonal research institute, Jhansi. Five flavonoid glycosides, kaempferol-3-O-glucoside, kaempferol-3-O-diglucoside, quercetin-3-O-glucoside, quercetin-3-O-diglucoside and quercetin-3-O-diglucoside-3'–O-xyloside have been isolated and characterised.

EXPERIMENTAL

Air-dried fronds (250g) of *A. solenopelis* was extracted with MeOH : H_2O (1:1 v/v; 1.5 lit.) under reflux for 6 hours. The extract was subjected to low Pressure column chromatography on sephadex LH - 20 (2.5 × 13 cm. LH 20 Column) using 20% methanol to 100% methanol to separate the extract into four bands. The flovonoid glycosides were found in bands 3 and 4. Two dimensional paper chromatographic work - up of each band was performed on whatmann 3 filter paper **[11]** using t-butanol : acetic acid : water (3:1:1) to purify the flavonoids. UV- Vis spectroscopy and shift regents studies were performed following the Method of Mabry et al, **[12]**. Acid hydrolysis of glycoside was carried out with 5% ethanolic HCL and the aglycones were identified by comparison with authentic samples. Suger moties were identified by paper chromatography in Comparison with outhentic sugars and also by GLC as trimethylsilyl ether on 3% SE-52 on acid - washed chromosorb W (column Temperature 180^0). For GLC Varian - 3400 series (FID) was used.

RESULTS AND DISCUSSION

On the basis of 2d PC, R_F values, UV-vis spectral data with shift reagent studies, acid hydrolysis, TLC studies of glycones with authentic samples and by GLC studies (Table 1) the isolated compounds were identified as the following five flavonoid glycosides : Kaempferol-3-O-glucoside, Kaempferol-3-O-diglucoside, quercetin-3-O-glucoside, quercetin-3-O-diglucoside and quercetin-3-O-diglucoside-3'-O-xyloside.

Kaempferol-3-O-glucoside has been isolated from *Ahtyrium filix-famina* **[13]** and from *Acystopteris Japonica* **[14]** and *Hypodematium crenatum* and *H. Fauriei* **[15]**. Quercetin-3-O-glucoside has been reported from *Onoclea sensibilis* **[14].** In the present investigation, presence of kaempferol and quercetin-3-O-glucoside in the advanced fern, *Athyrium solenopteris* (Kunze) may be due to the fact that complexity of flavonoids will increase with the morphogenetic advancement. Presence of glucose moiety in all the five flavonoid glycosides indicates that glucose is the primitive glycosylating sugar. As a result of photosynthesis and primary metabolism one would expect that glucose to be a primitive glycosylating moiety **[16].** Flavonoid glucosides are commonly distributed in *Athyriod* ferns. It is clear that several *Athyroid* ferns possess kaempferol-3-O-glucoside and quercetin-3-O-glucoside and hence these compounds may be considered as chemotaxonomic markers for the identification of *Athyrium solenopteris.*

Table 1. UV - visible spectral and chromatographic data of flavonoid glycosides of *Athyrium solenopteris*

Sl. No.	Compd.	UV-Visible spectra (nm) MeOH	NaOMe Immediately	NaOMe After 10 min	$AlCl_3$	$AlCl_3$ /HCl	NaOAc	R_f values TBA : HOAc : H_2O (3:1:1)	HOAc (15%)	Sugar (s) present
1	Kaempfero l-3-O-glucoside	265, 343	283*, 432*	Decom.	265, 303(sh) 356, 419	265 (sh), 303 (sh), 354, 419	273, 303, 379	0.70	0.55	Glusose
2	Kaempferol - 3-O-diglucoside	264, 300 (sh), 346	275*, 318*, 401*	Decom.	272, 303, 356, 383	272, 301, 346, 380	272, 307, 388	0.62	0.66	Glusose
3	Quercetin - 3-O-glucoside	255, 262 (sh), 293, 356	271*, 323* 404*	Decom.	358, 426	274, 358, 426	273, 320, 385	0.55	0.43	Glusose
4	Quercetin - 3-O-diglucoside	256, 264 (sh), 300 (sh), 356	272*, 315* 412*	Decom.	273, 302 (sh), 365 (sh), 430	265, 300 (sh), 360, 394 (sh)	273, 320 (sh), 405	0.50	0.61	Glusose
5	Quercetin - 3-O-diglucoside-3'-O-glucoside	256, 266 (sh), 300 (sh), 357	273*, 310* (sh), 416*	Decom.	266, 300, 364, 392 (sh)	266, 300, 362, 390 (sh)	272, 322, 405	0.35	0.56	Glusose

*increase in intensity

CHAPTER - 4

Chemical Investigation of *Desmodium gangeticum* Dc.

INTRODUCTION

Desmodium gangeticum Dc. [17-19] belongs to family Leguminosae which is known as 'Sarivan' in Hindi. It is found almost throughout in India, ascending to 5000 ft. in Himalayas. The plant is considered to be antipyretic and anticatarrhalic. Its root is hot and bitter and useful in the treatment of chronic fevers, nausea, vomiting, cough and snake bite. Its seeds are used as febrifuge. In the present we report the isolation and identification of a novel bioactive flavonol glycoside, 3, 5, 7, 4' – Tetrahydroxy – 8 – methyl flavone – 3 – 0 – α – L – rhamnopyranosyl – (1 → 6) – O – β – D – galactopyranoside from the stems of this plant.

EXPERIMENTAL

M.ps. are uncorrected. UV spectra were determined in MeOH and IR spectra recorded in KBr discs. ^{1}H NMR spectra were run at 300 MHz using TMS as internal standard and $CDCl_3$ as solvent. ^{13}C NMR spectra were measured at 90 MHz using DMSO – d_6 as solvent.

Plant Material : The stems of *desmodium gangeticum* were collected around the Bundlekhand region and taxonomically authenticated by department of Botany, Pt.J.L.N. (P.G.) College Banda (U.P.).

Extraction and isolation : The air dried and powdered stems (3.5 kg) of the

plant were extracted with 95% EtOH in a Soxhlet extractor. The total ethanolic extract was concentrated under reduced pressure. The concentrated ethanolic extract was successively partitioned with petroleum ether (60-80^0), benzene, chloroform, ethyl acetate, acetone and methanol. The methanol fraction of ethanolic extract of the plant was concentrated to brownish viscous mass, which was subjected to TLC examination using $CHCl_3$:MeOH : H_2O (15 : 9 : 4) as eluent and I_2 vapours as visualising agent, gave two spots, indicating it to be mixture of two compounds **1** and **1a** which were separated by TLC and purified by column chromatography over Si-gel G. The quantity of compound **1a** was found in very small amount therefore it was not possible to examine it further. Compound **1** was further purified by column chromatography which was found to be homogeneous on TLC examination. It was crystallised from methanol as yellowish needles compound (1.05 g). It had m.p. 279-281^0; $C_{28}H_{32}O_{15}$, $[M]^+$ 608 (FABMS) (Found : C, 55.26; H, 5.25. Calcd. : C, 55.24; H, 5.29%). IR (KBr) ν_{max} 3420, 2945, 2935, 1635, 1620, 1520, 1205, 860 cm–1; UV λ_{max} (MeOH) 274, 331, 379; (+NaOMe) 262, 285, 427, (+ NaOAc) 272, 329, 417; (+ $AlCl_3$) 268, 365, 379; (+$AlCl_3$/HCl) 270, 365, 404; (+NaOAc/H_3BO_3) 271, 326, 412 nm. It formed a nona acetyl derivative **3**, m.p. 183–184^0, $C_{46}H_{50}O_{24}$, $[M]^+$ 986 (Found:C, 55.95; H 5.09. Calcd. : C, 55.98; H 5.07%). ^{1}H NMR (300 MHz, CDCl3) δ 6.98 (2H, d, J 8.5 Hz, H–3', 5'), 7.78 (2H, d, J 8.5 H, H–2' 6'), 2.34 (3H, s, 5–OAc), 6.80 (1H, s, H–6), 2.85 (3H, s, 7–OAc), 2.12 (3H, s, 8–Me), 2.41(3H, s, 4'–OAc), 5.74 (1H, d, J 8.0 Hz, H–1"), 2.92 (3H, s, 2"–OAc), 2.95(3H, s, 3" – OAc), 2.98 (3H, s, 4"–OAc), 3.82 –

4.31 (6H, m, protons of galactose unit), 4.36 (1H, d, J 7.6 Hz, H–1'''), 2.80 (3H, s, 2'''–OAc), 2.14 (3H, s, 3'''–OAc), 2.20 (3H, s, 4'''– OAc), 1.18 (3H, m, Me – 6'''), 4.75–5.35 (4H, m, protons of rhamnose unit). ^{13}C NMR (90 MHz, DMSO – d_6) table 1. $[M]^+$ 608, m/z 462 (aglycone ion), 272, 271, 167, 166, 138, 134, 133.

Acid hydrolysis of compound 1 : Compound **1** was hydrolysed by refluxing with 10% H_2SO_4 for 6h at 100^0. On cooling, the aglycone **2** was crystallised from MeOH as yellowish plates which was identified as 3, 5, 7, 4' – tetrahydroxy – 8 – methyl flavone, $C_{16}H_{12}O_6$, m.p.286–287^0, $[M]^+$ 300 (EIMS), m/z 272, 271, 167, 166, 138, 134, 133 (Found : C, 64.03; H, 4.0, Calcd. : C, 64.01; H 4.02%); UV λ_{max}(+ MeOH) 276, 332, 380; (+NaOMe) 265, 284, 428; (+$AlCl_3$) 269, 366, 434; (+$AlCl_3$/HCl) 268, 362, 405; (+NaOAc) 273, 330, 418; (+NaOAc/H_3BO_3) 273, 328, 411 nm; ^1H NMR (300 MHz, $CDCl_3$) of **4** at δ 2.12 (3H, s, 8 – Me), 6.80 (1H, s, H–6), 7.78 (2H, d, J 8.5 Hz, H–2', H–6'), 6.98 (2H, d, J 8.5 Hz, H–3', H–5'), 2.38 (3H, s, 3–OAc), 2.34 (3H, s, 5–OAc), 2.85 (3H, s, 7–OAc), 2.41 (3H, s, 4'–OAc).

The aqueous hydrolysate was neutralised with $BaCO_3$ and $BaSo_4$ filtered off. The filtrate was concentrated and subjected to PC examination on Whatman filter paper no. 1 using n-BuOH : HOAc : H_2O (4 :1 :5, v/v) as solvent and aniline hydrogen phthalate as detecting agent yielded D–galactose (R_f 0.15) and L–rhamnose (R_f 0.36).

Permethylation and hydrolysis of the compound 1 : Compound 1 (15 mg) was treated with CH_3I (2 ml) and Ag_2O (20 mg) in DMF (5 ml) at room temperature for 2 days. The contents were filtered and the residue was treated with EtOH (3 ml). The syrupy residue was hydrolysed with 10% H_2SO_4 for 6–7 h. After usual work up, it yielded methylated aglycone, identified as 3–hydroxy–5, 7, 4' – trimethoxy – 8 – methyl flavone and methylated sugars, were identified as 2, 3, 4 – tri – O – methyl – L – rhamnose and 2, 3, 4 – tri – O – methyl – D – galactose.

Table 1. ^{13}C NMR (90 MHz, DMSO–d_6) of compound 1

Atom	δ–Value	Atom	δ–Value
C–2	148.3	C–5'	115.7
C–3	133.4	C–6'	129.4
C–4	176.6	C–1"	102.9
C–5	159.1	C–2"	71.4
C–6	98.4	C–3"	73.2
C–7	161.2	C–4"	68.1
C–8	104.3	C–5"	73.8
C–9	152.8	C–6"	65.7
C–10	104.6	C–1"'	101.2
C–1'	122.9	C–2"'	70.3
C–2'	129.7	C–3"'	70.6
C–3'	115.8	C–4"'	72.1
C–4'	159.2	C–5"'	58.3
		C–6"'	17.9

RESULTS AND DISCUSSION

The methanol fraction of stems of the plant afforded a novel bioactive compound **1**, m.p. 279 – 281^0, $C_{28}H_{32}O_{15}$, $[M]^+$ 608 (FABMS). It responded Molisch and Shinoda tests **[20]** showing its flavonoidal glycosidic nature. It was crystallised as a yellowish needles compound. Its I R spectrum showed absorption bands at 3420 (–OH), 2945 (C – H), 1635 (>C = 0), 1620 (aromatic ring system) and 1520, 1205, 860cm^{-1}. Its UV spectrum with various shift reagents suggested the presence of free hydroxyl groups at C–5, C–7and C–4' positions **[21, 22]**.

The compound **1** on acid hydrolysis with 10% H_2SO_4 yielded an aglycone **2**, m.p. 285 – 286^0, $C_{16}H_{12}O_6$, $[M]^+$ 300 (EIMS) and sugar moieties, identified as D–galactose and L–rhamnose. A retro-Diels-Alder fragmentation patterns resulted in the formation of ions at m/z 272, 271, 167, 166, 138, 134, 133. These results supported the presence of a methyl group and two hydroxyl groups in ring A and one hydroxyl group in ring B.

When aglycone **2** was treated with Ac_2O/pyridine it formed tetra acetyl derivative, **4**, m.p. 186–187^0, $C_{24}H_{20}O_{10}$, and $[M]^+$ 468 (EIMS). The ^{1}H NMR spectrum of **4** showed two ortho coupled doublets at δ 7.78 (2H, J 8.5 Hz), 6.98 (2H, J 8.5 Hz) corresponding for H –2', 6' and H–3', 5' respectively, confirming ring B to be symmetrically substituted. A singlet of one proton intensity at δ 6.34 was assigned to C–6 position. A singlet at δ 2.12 integrating for three protons was assigned to methyl group at C–8 position. Resonance signals at δ 2.34 (3H, s, 5

– OAc), 2.38 (3H, s, 3 – OAc), 2.84 (3H, s, 7 – OAc) and 2.41 (3H, s, 4' – OAc) were indicative of four hydroxyl groups in aglycone **2**. The compound **2** was further identified as 3, 5, 7, 4' – tetrahydroxy – 8 – methyl flavone with reported literature **[23, 24].**

The compound **1** was treated with Ac_2O/ pyridine at 120^0 for about 12 h and gave nona acetyl derivative **3**, m.p. 183–184^0, $C_{46}H_{50}O_{24}$ and $[M]^+$ 986. The ^1H NMR of **3** showed signals for anomeric protons at δ 5.74 (1H, d, J 8.0 Hz, H–1") and 4.36 (1H, d, J 7.6 Hz, H–1"'), assigned to D–galactose and L–rhamnose respectively. A complex signal at δ 1.18 was assigned to rhamnosyl methyl group.

Permethylation **[25]** of **1** followed by acid hydrolysis gave permethylated aglycone, identified as 3–hydroxy–5, 7, 4'– trimethoxy – 8 – methyl flavone which confirmed that –OH group at C – 3 position of the aglycone was involved in glycosylation. The methylated sugar which were identified as 2, 3, 4 – tri – 0 – methyl – D – galactose and 2, 3, 4 – tri – 0 – methyl – L – rhamnose according to Petek **[26]** showed that the C–1"' of L – rhamnose was linked with C – 6" of D – galactose and C – 1" of D –galactose was attached with C–3 position of the aglycone and also showed the interlinkate (1 → 6) between the sugars.

Enzymatic hydrolysis of the compound **1** by Takadiastase liberated L – rhamnose and 3, 5, 7, 4' – tetrahydoxy – 8 – methyl flavone – 3 – 0 – β – D – galactopyranoside as pyroaglycone which confirmed the presence of α – linkage between L – rhamnose and D – galactose. The proaglycone on further hydrolysis

CHAPTER - 5

Cyanolipids

I. INTRODUCTION

Cyanolipids are a new class of plant lipids which are found, often in copious amounts, only in the seed oils of Sapindaceae plats and probably play an important role in the biochemistry of these plants. Although the occurrence of cyanolipid materials has been suspected for many years **[27, 28]** due primarily to the cyanogenic property of kusum seed oil, only recently **[29-33]** have their structures been determined and the existence of this class of lipids acknowledged **[34, 35]**.

The unifying structural feature of these cyanolipids is that they are all based on the same branched, five-carbon nitrile skeleton, although the double bond position and the number and location of hydroxyl groups are not the same. Two of the four known cyanolipids do not fit in the "diol lipid" category **[36]** because they are monoesters, and cyanolipids as a group cannot be labelled "cyanogenic lipids" because two of them do not liberate HCN. The unique structures of this new class of lipids enable them to undergo reactions (as well as to be derived from biogenetic pathways) that are distinctive from any other lipid class.

Some earlier reports on unidentified "cyanogenic glycosides" may, in fact, deal with cyanolipids. This facet remains unexplored. In addition, it is easy to imagine how investigators who were not specifically searching for cyanolipids would not have detected them; in years past, cyanolipids have been repeatedly overlooked in seed oils containing them in large amounts.

Being a relatively new development, the cyanolipid field does not possess a

large body of literature as do other lipid subdivisions. In order to build a firm basis for further discussion, a brief synopsis of cyanogenesis is provided, particularly as it relates to result of kusum oil research obtained prior to the actual structure proof of cyanolipids. Cyanolipids appear to occur only in Sapindaceae plants; therefore, a sampling of pre-cyanolipid literature describing research on some of the more important plants of this family (in addition to kusum) has also been made.

Beacause these lipids undergo distinctive and interesting reactions, detailed information and some experimental procedures were deemed beneficial and have been included. Results of NMR analysis are thoroughly explored because this technique has proven most definitive in sorting out the various cyanolipid structures.

Biosynthetic studies on cyanolipids are just beginning to yield returs, and a summary of these findings is also presented.

Structural diagrams in the text and especially in figures are displayed in the most convenient manner, and no stereochemical implications are intended.

A. Cyanogenesis in Plants

Plants which are capable of yielding HCN by hydrolysis of chemical materials they produce have long been known. This process, termed cyanogenesis, can occur it damaged plant tissues by endogenous enzymic action, or by the action of chemical hydrolyzing agents on the plant materials. Numerous examples of chemi-

cal hydrolysis and demonstrated by the adverse effects ingestion of certain of these plants can have on man and animals.[37, 38]

Cyanogenesis has historically been associated exclusively with the presence of cyanogenic glycosides in plant tissues. Even though cyanogenic activity has been observed in tissue from 800 to 1000 different plant species, representing more than seventy families [39, 40] the actual chemical entities responsible for this cyanogenicity are known in scarcely 50-60 species. In fact, Paris [41] in 1963, commented that in spite of the fact that many plants released HCN on treatment with enzyme or acid, it was by no means a certainty that they contained cyanogenic glycosides; he thereby inferred that other types of cyanogenic materials might be responsible for this activity in some cases. We now know that certain cyanolipids, which are not glycosides, do, indeed, release HCN.

1. *Schleichera Trijuga* (Kusum) Seed Oil

Up to the time that the true nature of cyanolipids was discovered, *Schleichera trijuga* (kusum) was the sapindaceous plant that had been most thoroughly investigated, especially its seed lipids. Kusum is a tree found almost exclusively in India and adjacent areas. Kusum seed oil, which has been an item of commerce there for some time, is described by Eckey [42] as being used variously as a medicinal oil, a hair dressing, an edible oil and a raw material for soap production.[43] The cyanogenic property of kusum oil has long been recognized and documented [27, 28] and numerous reports describing its fatty acid and triglyceride composition have

appeared.[44–50] Oleic acid was usually reported [42, 46, 47] to be the major constituent of this oil, ranging from 40 to 62%. It was also generally agreed that kusum oil had little or no linoleic or linolenic acids, but that relatively large amounts (20-25%) of arachidic acid were observable by methods available to the investigators.

More recently, GLC has provided ample confirmaiton of these earlier conclusions by analysis of mixed methyl esters prepared from kusum oil.[42, 50] In addition, Dhar [45] actually isolated and characterized arachidic acid from saponified kusum oil. However, Basu [51] recently published a somewhat sketchy report showing a GLC analysis of kusum oil methyl esters that disagrees with all other published data. He reports 50% linoleic acid, 22% oleic acid and only 1% of C_{20} acids. The reason for this wide variation from generally accepted values is unclear.

The first real, though unsuccessful, attempt to clarify the structure of cyanogenic materials in *Schleichera trijuga* seed oil was made by Neogi and Adhicari [52]; the abstract of their paper states that HCN in kusum oil was derived from amygdalin and an enzyme giving the test of emulsin. The authors claimed to have isolated both the glycoside and the enzyme. However, anygdalin has not subsequently been found in kusum seeds or its derived oil, and since this report has not been acknowledged by later investigators, its significance must be discounted.

Because of persistent problems associated with processing [53] and use [52] of this oil, a patent [54] was granted for a process devised to reduce the cyanogenic properties and also the "nonglycerol alcohol" content of the oil. It involved

treating the oil at 120°C with zinc oxide, water and sodium hydrosulfite ($Na_2S_2O_3.2H_2O$). Although these investigators recognized the occurrence of nonglycerol esters [53, 55] in kusum oil, they apparently did not suspect a conection between these esters and the oil's cyanogenic behavior.

At about the same time, Kundu and Bandyopadhyay [56] arrived at the other half of the enigma, namely that HCN liberated by kusum oil was not derived from cyanogenic glycosides because they were not present in the oil. By virtue of the fact that steam distillation, although part of a standard method [57] to quantitatively remove cyanogenic glycosides as HCL, failed to eliminate the cyanogenic properties of kusum oil the authors reasoned (and later demonstrated [58] by TLC) that cyanogenic glycosides were indeed absent. However, the investigators still did not link the absence of a glycoside, the presence of nonglycerol esters, and cyanogenesis in kusum oil. Thus, although the structures proposed by Kundu and Bandyopadhyay [56, 58, 59] were incorrect, they provided the first conclusive evidence and documentation that cyanogenicity in kusum oil was the direct result of a lipid -based mateiral.

B. Properties of Sapindaceous Plants

A number of sapindaceous plants other than kusum have been quite thoroughly investigated [42] because of economic interest and some possess interesting properties or constituents. Among these plants are *Blighia sapida,* (akee) fruit, a major food of Jamaica [60] which contains toxic polypeptides when immature [61] *Sapindus* seed, which produces saponins [62–64] in large quantities, is used in soap manufacture; nothing particularly unusual was noted about its oil [65] however.

Paullinia cupana seed is ground and used extensively in Brazil to brew a coffee-or tea-like drink called guarana [66]. Edible fruits and seed oils [42] (which contain large amounts of C_{20} acids) [67, 68]) are derived from *Nephelium lappaceum* and *N. mutabile*.

Seeds of *Koelreuteria paniculata* (an ornamental) reportedly are insecticidal to mosquito larvae [69] and extracts of *sapindus saponaria* and *Paullinia fuscescens* plants exhibit toxicity to leaf feeding insects.

Seeds oil of *Ungnadia speciosa* (mexican buckeye) was reported to contain a cyanogenic glycoside [70] and to produce certain unpleasant effects, likely caused by HCN, when the oil was eaten as a salad oil. The seeds themselves were classified as poisonous. Apparently this glycoside has not been characterized.

Dihydrosterculic acid cis.9,10-methyleneoctadecanoic acid was shown to

be a conspicuous component (17%) of glycerides of *Euphoria longana* seed oil [71]. This is the only known report of cyclopreoanoid acids occuring in sapindaceous plants, but they may also be found in Litchi chinensis seed oils [72].

Other reports [61, 73–75] deal with miscellaneous features of various sapindaceous plants.

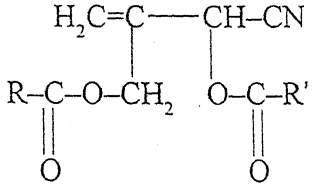
One particularly noticeable feature of sapindaceae seed oils is that a majority of them comprise large amounts (up to 32% in *Paullinia meliaefolia* [67]) of C_{20} fatty acids; [67, 76–78] these may be saturated, unsaturated (primarily monoenoic) or a mixture of both. One should exercise caution when interpreting Sapindaceae seed oil compositions which were obtained without benefit of GLC because these analysis were often incomplete due to difficulties in determining the C_{20} acid content.

II. STRUCTURAL FEATURES, DISTRIBUTION AND BIOSYNTHESIS

A. Cyanolipid Structures

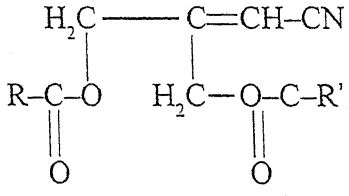
Progress in cyanolipid identification began with reports [55, 56, 58, 59] on kusum oil by workers in India, even though their data and conclusions were misleading. At about the same time, Mikolajczak and colleagues, in a series of publications [29–33, 79] described the detection, isolation and structure proof of four types of cyanolipids having different but closely related structures. As a result, the earlier work that had demonstrated the presence of nonglycerol esters, the absence of glycosides and the cyanogenicity of kusum oil was all meshed together [29].

Two of the newly discovered materials (I, II) were shown to be long chain fatty acid diesters of 1-cyano-2-hydroxymethylprop-2-ene-1-ol (structure I) and 1-cyano-2-hydroxymethylrprop-1-ene-3-ol (structure II).



I

SCHEME 1



II

SCHEME 2

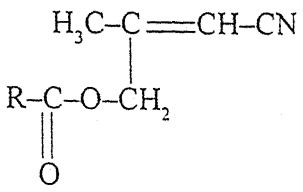
Table 1. Fatty acid content of cyanolipids

	Fatty acid chain length degree of unsaturation											
Cyanolipid	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	22:1
I_a	–	1	1	1	16	–	–	24	49	–	4	4
I_f	2	4	1	1	33	3	–	29	22	Trace	2	3
$II_{a,b}$	3	6	1	2	43	4	–	12	24	Trace	2	3
II_d	–	6	–	1	24	6	1	3	55	1	Trace	3
III_c	–	1	–	Trace	3	1	–	6	84	2	1	2
III_d	–	1	–	Trace	3	2	–	2	86	2	–	4
IV_e	–	–	–	–	–	–	–	28	72	–	–	–

[a]From *Cordia verbenacea* **[32]**
[b]From *Schleichera trijuga* **[29]**
[c]Includes 1% of C12:0
[d]From *Koelreuteria paniculata* **[31]**
[e]From *Stocksia brahuica* **[30]**
[f]From *Ungnadia speciosa* **[33]**

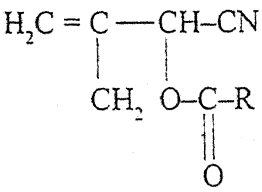
Cyanolipid I is cyanogenic, but II is not. From certain chemical data there seems to became preference for the cyanohydrin hydroxyl group of I to be acylated with C_{18} acid. However, ordinary analytical or spectral data do not distinguish between R and R' and both should be considered to be mixtures of long chain (C_{12} to C_{22}) fatty acids containing major amounts of C_{20} acids. (see table 1.)

The other two cyanolipids isolated and characterized (III, IV) were also fatty acid esters, but of the monohydroxy nitriles, 1-cyano-2-hydroxymethylprop-1-ene (structure III, which is not cyanogenic) and 1-cyano-2-methylprop-2-ene-1-ol (structure IV, which is cyanogenic).



III

SCHEME 3



IV

SCHEME 4

The R-groups of III and IV are again long chain fatty acids, but in contrast with the diol esters, are almost exclusively C_{20} saturated (arachidic) and C_{20} monoenoic acids. Only minor amounts of other chain lengths are observed as outlined in Table 1. These data mean that the fatty acid composition of any particular cyanolipid based on these alcohols is likely to vary considerably, depending on the composition of triglycerides with which it is associated and, hence on what plant produces it.

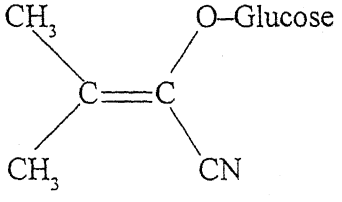
Cyanolipids I **[32]** and IV **[33]** are optically active and both give plain positive optical rotatory disperson curves. They (I and IV) are cyanohydrins with the cyanohydrin hydroxyl group esterified and they are also β,γ-unsaturated nitriles; II and III are simply α,β-unsaturated nitriles and hence have no chiral center. An important point to realize concerning the structures of these four cyanolipids is that unlike I and IV, cyanolipids II and III are not cyanohydrin esters, hence they do not liberate HCN, and do not respond to color tests normally associated with HCN detection.

All four cyanolipid alcohols are based on the same five-carbon skeleton. No work concerning the absolute configurations of I and IV has been reported.

B. Similar Structures

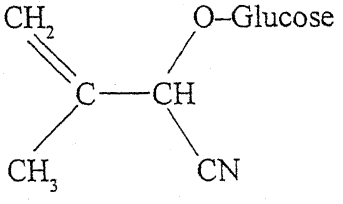
1. Naturally Occuring

While cyanolipid structures I, II, III and IV were not known prior to the investigations of Mikolajczak et al., **[29, 30, 78, 79]** and Seigler et al., **[32, 33]** a cyanogenic glycoside isolated from two species of *Acacia* **[80, 81]** and named acacipetalin, was identified as V. Recently structure V was shown to be incorrect **[82]** and acacipetalin actually has structure VI instead.



V

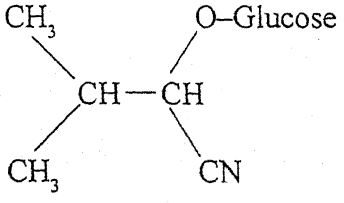
SCHEME 5



VI

SCHEME 6

The hydroxynitrile moiety of VI is identical (except possibly for stereochemistry, which is unknown) with that of cyanolipid IV. In addition, a new cyanogenic glycoside, named dihydroacacipetalin, was recently isolated from *Acacia sieberiana,* a legume and also from *Heterodendron oleaefolium,* a Sapindaceae **[83]** and was shown to have structure VII. The hydroxynitrile moiety of VII is the same as that (may have different stereochemistry) of the product one obtains by hydrogenation of cyanolipid IV.



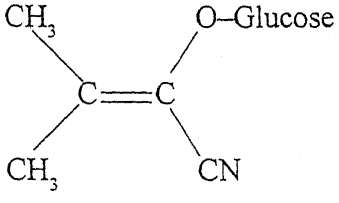
VII

SCHEME 7

B. Similar Structures

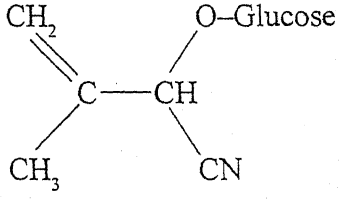
1. Naturally Occuring

While cyanolipid structures I, II, III and IV were not known prior to the investigations of Mikolajczak et al., **[29, 30, 78, 79]** and Seigler et al., **[32, 33]** a cyanogenic glycoside isolated from two species of *Acacia* **[80, 81]** and named acacipetalin, was identified as V. Recently structure V was shown to be incorrect **[82]** and acacipetalin actually has structure VI instead.



V

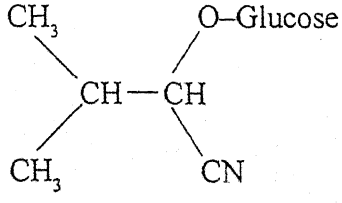
SCHEME 5



VI

SCHEME 6

The hydroxynitrile moiety of VI is identical (except possibly for stereochemistry, which is unknown) with that of cyanolipid IV. In addition, a new cyanogenic glycoside, named dihydroacacipetalin, was recently isolated from *Acacia sieberiana,* a legume and also from *Heterodendron oleaefolium,* a Sapindaceae **[83]** and was shown to have structure VII. The hydroxynitrile moiety of VII is the same as that (may have different stereochemistry) of the product one obtains by hydrogenation of cyanolipid IV.

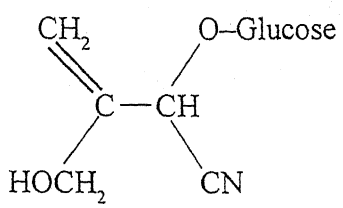


VII

SCHEME 7

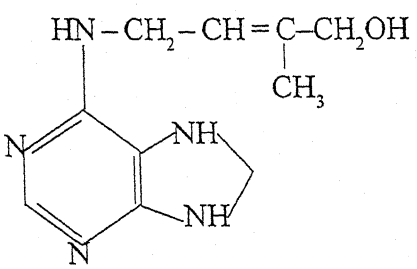
The dihydroxynitrile moiety of cyanolipid I has been isolated (stereochemisty may be different) in the form of the cyanogenic glycoside, cardiospermin (VIII), from vegetative parts of *Cardiospermum hirsutum* plants. The interesting feature of this observation is that *C. Hirsutum* **[84]** as well as C *halicacabum* **[78]** seed oils contain cyanolipid I as a major component (about 50%). Cardiospermin has also been isolated from leaves and twigs of another Sapindaceae plant, *Heterodendron oleaefolium* **[83]**.

The hydroxynitrile moiety of III has been synthesized and converted to a natural growth regulator, zeatin (IX), **[85]** where the nitrile occurs in a reduced form as a secondary amine derivative.



VIII

SCHEME 8



IX

SCHEME 9

Finally, a dihydroxy acid, isomeric with the acids one should get by hydrolysis the cyano groups of the dihydroxynitrile moieties of I and II, has been isolated from the alkali hydrolyzate of an antibiotically active glycoside from *Tulipa gesneri* (Liliaceae).

2. Synthetic

The cyanohydrin moiety found in cyanolipid IV has been prepared synthetically and then was oxidized with MnO_2 in methanol to methyl methacrylate. Synthetic roxynitriles that are structurally isomeric with those present in III and IV are known **[86–88]**. The dihydroxynitriles on which cyanolipids I and II are based have yet been synthesized; perhaps they are too unustable to exist as the free alcohols.

C. Distribution of Cyanolipids

Cyanolipids have thus far been found only in seed oils of sapindaceous plants; **[29–31, 33, 75, 84, 89, 90]** however, not all sapindaceous seeds contain cyanolipids.

Even though oils from plants of other families have been screened for cyanolipids, none have been detected **[75]**. Ironically, the plant from which cyanolipids (I) were first isolated and properly characterized had been identified as *Cordia verbenacea,* a borage **[32, 79]**. Later work, however, indicates that the identity of these seeds is questionable and that they are probably from a species belonging to the Sapindaceae **[91]**, not *Cordia verbenacea* as reported. Table 2 lists those seed oils for which quantitative determinations of their cyanolipids have been made.

Schleichera trijuga (kusum) seed oil yields the largest total amount of cyanolipid, but seed oils of some *Koelreuteria, Cardiospermum* and *Paullinia* species are close behind. Of course, Seeds from different accessions may display differing amounts of cyanolipids depending on such variables as growing location, seed maturity and storage conditions.

Table 2. Cyanolipid content of seed oils

Species	Cyanolipid %				Ref.
	I	II	III	IV	
Allaphyllus edulis	30	Trace	–	–	78
Cardiospermum halicacabum	49	6	–	–	78
Cardiospermum hirsutum	50	–	–	–	84
Cordia verbenacea[a]	35	–	–	–	78
Koelreuteria paniculata	–	25	17	–	78
Koelreuteria paniculata	–	25	31	–	84
Nephelium lappaceum	–	21	–	–	78
Paullinia meliaefolia	53	–	–	–	78
Paullinia tomentosa	26	–	–	–	84
Sapindus drumnondii	–	28	–	–	84
Sapindus mukorossi	–	13	–	–	78
Sapindus utilis	–	32	–	–	84
Schleichera trijuga (kusum)	58	6	–	–	29
Stocksia brahuica	–	Trace	35	–	78
Ungnadia speciosa	–	Trace	Trace	29	78
Ungnadia speciosa	–	–	Trace	33	84
Urvillea uniloba	19	4	–	–	78

[a]Plant identification is probably erroneous.[91]

Cyanolipids have recently been detected in a number of Sapindaceae plant seeds by applying Seigler's [84] NMR technique. Which cyanolipid (s) were actually present was determined by the presence or absence of appropriate NMR signals, but amounts were not reported [75]. No new cyanolipids were found. A listing of these species along with the identity of the cyanolipids in their seed oils is presented in Table 3.

An additonal Sapindaceae species, *Cardiospermum canescens,* also is reported to contain cyanolipids [92].

Some interesting points which evolve from data in Table 2 and 3 deserve comment: (a) cyanolipid I is by far the most abundant and usually occurs either alone or with a minor amount of II; (b) cyanolipid III appears, for the most part, in combination with cyanolipid II; (c) no oil examined to date has contained all four cyanolipids: (d) cyanolipid IV has been detected in only one seed oil, that of *Ungnadia speciosa*; and (e) members of a particular genus usually produce the same cyanolipid(s).

The conclusion that cyanolipids do not occur universally in Sapindaceae seed oils is documented by Table 4, a compilation of those Sapindaceae seed oils in which cyanolipids were not detected [75, 78, 84]. However, certain of these species, designated by footnote b, gave strongly cyanogenic oil-free meals. From these results, one concludes that the presence of cyanolipids in a particular seed oil is not a prerequisite for cyanogenicity in the seed itself; these HCN-releasing materials

that are not extracted from the seed with lipid solvents have not been investigated.

Different accessions of seed from a particular species may at times yield entirely opposite results instead of just variations in amounts of cyanolipids. For example, in Table 4, *Paullinia cupana* and *Sapindus Saponaria* seed oils are listed as being devoid of cyanolipids while Table 3 reports that they do contain cyanolipids. This apparent anomaly is probably caused simply by variations between different batches of seed and should not be cause for concern; similar irregularities crop up frequently in research on plant materials.

The search for cyanolipids in seeds of other plants (not Sapindaceae) has been extended by Seigler and Kawahara **[75]** to include representatives of four families (shown in Table 5) which are closely allied botanically to the Sapindaceae. None of these seed oils examined gave any indications of the presence of cyanolipids.

Table 3. Cyanolipids Detected by NMR Analysis of Sapindaceae Seed Oils[a]

Species	Cyanolipid(s) detected
Alectryon carinatus	I
Alectryon excelsum	I
Alectryon subcinereus	I
Alectryon tomentosus	I
Allophyllus concina	I, II, III
Allophyllus occidentalis	I
Cardiospermum grandiflorum	I
Cardiospermum microcarpum	I
Dipterodendron elegans	II
Jagera pseudorhus	I
Koelreuteria henryi	II, III
Lecaniodiscus cupanioides	II, III
Pappea cappensis	II
Paullinia bracteosa	I
Paullinia cupana	I
Paullinia cururu	I
Paullinia elegans	I
Paullinia hystrix	I
Paullinia trichornis	I
Podonephelium homei	I
Sapindus saponaria	II
Serjania atrolineata	I
Serjania cissifolia	I
Serjania goniocarpa	I
Serjania punctata	I
Serjania trifolia	I
Serjania triquetra	I
Toulicia guaianensis	II
Urvillea ulmacea	I

[a]Taken from seigler and Kawahara [75] only species not listed in Table 2 are given here.

Table 4. Sapindaceae seed oils containing no cyanolipids[a]

Species	Species
Aphanea senegalensis	*Litchi chinensis*[a]
Blighia sapida	*Macphersonia madagascariensis*[b]
Cardiospermum integerrimum[b]	*Maytayba glaberrima*
Cupania americana	*Maytayba oppositifolia*
Cupania costaricensis[b]	*Paullinia caloptera*
Cupania glabra[b]	*Paullinia capreolata*[b]
Cupania papillosa	*Paullinia cupana*[b,d]
Deinbollia grandiflora	*Pseudima sp.*[b]
Deinbollia pinnata	*Sapindus saponaria*[d]
Dodonea lanceolata	*Serjania rhombea*
Dodonea viscosa	*Talisia floresii*
Euphoria longana	*Thouindium decandrum*
Exothea paniculata[b]	*Xanthoceras sorbifolia*
Harpullia zanguebarica	

[a]Taken primarily from Seigler and Kawahara [75]

[b]Oil-free meal is cyanogenic [75]

[c]See refs. [78] and [84]

[d]Other bathces of seed did contain cyanolipids, see Table 3.

Table 5. Related Plants Containing No Cyanolipids[a]

Species	Family
Billia columbianum	Hippocastanaceae
Billia hippocastanum	Hippocastanaceae
Melianthus major	Melianthaceae
Melianthus minor	Melianthaceae
Melianthus pectinatus	Melianthaceae
Meliosma matudae	Meliosmaceae
Staphylea trifolia[b]	Staphyleaceae

[a]Taken primarily from Seigler and Kawahara [75]. All are from Sapindales order. [b]see ref. [84]

D. Biosynthesis

Only a meager amount of research has thus far been reported concerning how these strange cyanolipids are produced in plants. Mikolajczak et al., [78] first pointed out that the structures of the hydroxynitrile portions of cyanolipids I-IV suggested that they might be derived from leucine. This hypothesis has been tested by Seigler and co-workers **[82, 93]** in *Acacia sieberiana* which, although not a member of the Sapindaceae; contains the same hydroxynitrile moiety as cyanolipid IV, and in *Koelreuteria paniculata* which elaborates cyanolipids II and III.

L-[U-^{14}C]-Leucine was by far the most efficacious precursor for the hydroxynitrile moiety in *Acacia* glycoside (VI). Similarly, leucine was an effective precursor for the hydroxynitrile moiety of cyanolipid III in *Koelreuteria paniculata* seed oil, but concurrent incorporation of the label into cyanolipid II was somewhat less **[93]**.

Some incorporation of L-[U-^{14}C]-valine into the *Acacia* glycoside (VI) hydroxynitrile residue was observed **[82]**. This incorporation likely occured by way of valine first being converted to leucine with acetyl-CoA. However, this route using valine should not yield labelled HCN as it does; to explain this result, the authors have proposed that randomization of the label into leucine occurred. Although additional experiments with other plant species are needed, it seems probable on the basis of evidence at hand that leucine is indeed, the precursor for the hydroxy and dihydroxynitrile portions of cyanolipids known to date.

At this time it is not known if any relationship exists between the cyanogenic glycoside, cardiospermin (VIII), which occurs in vegetative parts of *Cardiospermum hirsutum*, and cyanolipid I, which is found in seed oil of the same species. Similarly the occurrence in *Acacia* (a Leguminoseae) of a cyanogenic glycoside which is a derivative of the hydroxynitrile moiety in cyanolipid IV from *Ungnadia* (a Sapindaceae) is somewhat surprising, especially since cyanolipid IV occurs only in *Ungnadia* seed oil. An interesting aspect concerning cyanolipid IV is that it has been synthesized, and the investigator expects to use an incorporated radioactive label to trace the pathway that IV follows when fed to *Ungnadia seedlings* [94].

In discussing biosynthesis and how these strange types of materials accumulate in plant tissues, it is difficult to refrain from wondering why they are there. A convenient way to classify odd natural products is to label them "secondary metabolites" because their biochemical significance in the host plant is so obscure. A rational, oft-advanced possibility is that the plant accumulates these odd materials as storage facilities for carbon or, as exemplified by alkaloids and cyanolipids, nitrogen, until the need for these elements arises. Another theory of the significance of these materials is that they have no function in the biochemistry of the plant, but serve as potent insecticides and thus protect the plant. A third hypothesis is that these unusual compounds aid specific insects in locating a particular plant, either to feed, lay eggs or to somehow assist in the plant's reproductive cycle. Actually, rather impressive evidence has been accumulated that suggests not only that

all of these possibilites are valid to some extent, but also that secondary metabolities may be more actively involved in primary processes than was previously supposed.

Two recent mini-reviews elaborate on certain aspects of some secondary metabolites **[95, 96]**. One of these reports reveals that all of the cyanolipid IV found in *Ungnadia speciosa* seeds disintegrate within 3 days following germination; this finding strongly implies that in this one plant cyanolipids function as a storage facility for materials needed at this species point in its life cycle. Whether or not the same conclusion is applicable to other Sapindaceae seed oils is not known.

III. DETECTION, ANALYSIS AND ISOLATION OF CYANOLIPIDS

A. Detection and Analysis

1. Liberation of HCN

Various methods exist for qualitative detection of HCN released by cyanogenic plant materials and the method of choice is often a matter of personal preference. Three color tests are particularly suitable for use with oils containing cyanolipids. One is the picrate test **[32, 57, 97]** which depends on the ability of HCN to react with alkaline picrate saturated filter paper strips to produce isopurpuric acid **[98]**. The second test is based on formation of Prussian blue **[97]** and can be carried out as described by Seigler et al., **[32]**. A third procedure, based on the copper acetate-benzidine reaction, **[99]** apparently produces satisfactory results with kusum oil fractions **[56]** and also in the detection of small amounts of cyanogenic lipid adulterant in other vegetable oils and butter **[100]**.

While quantitative HCN determinations have not been routinely applied to cyanogenic lipids or to the oils containing them, a number of general methods **[101–104]** are available which might provide acceptable quantitation.

2. Infrared Spectral Properties

By itself, IR analysis of seed oils suspected of containing cyanolipids is not a particularly suitable detection method, but it can furnish valuable data when used in conjunction with other methods. Cyanolipids I and IV (cyanohydrin derivatives)

produce no truly characteristic IR bands as can be seen from fig. 1. The ester–C=O (1740 cm^{-1}) band of I is slightly braodened, however, weak, broad bands in the 920-965cm^{-1} and 1010 cm^{-1} regions, which probably reflect the $=CH_2$ grouping present in these cyanolipids, are essentially nonexistent in spectra of the whole seed oils. No –C≡N absorption is observed in the spectra of I and IV, because it is completely quenched by the proximity of the oxygen function **[105]**. However, this 2230 cm^{-1} band is prominent in spectra of II and III because, in these structures, the band's intensity is enchanced due to conjugation of the carbon-nitrogen triple bond with the double bond and due to the absence of an α-oxygen atom. In spite of this increased intensity, –C≡N absorption is not observed in the spectrum of *Sapindus mukorossi* seed oil which contains 13% of II, and is weak in the spectrum of *Nephelium lappaceum* oil containing 21% of II. Obviously, the relative intensities of this 2230cm^{-1} band in the IR spectra of II and III are directly related to the molecular weights of the two cyanolipids, which, in turn, are dependent primarily on the number of acyl moieties present. IR analysis therefore, would detect appreciable (>15%) amounts of cyanolipids II or III in a seed oil, but would be relatively undependable for oils containing I or IV or small percentages of II or III.

3. Thin-layer Chromatography

Among four types of cyanolipids each type can be adequately and quite conveniently distinguished from the other three and from triglycerides by separate TLC analysis in different solvent systems **[106]**. Using benzene with air-dried Silica Gel G plates, one obtains the

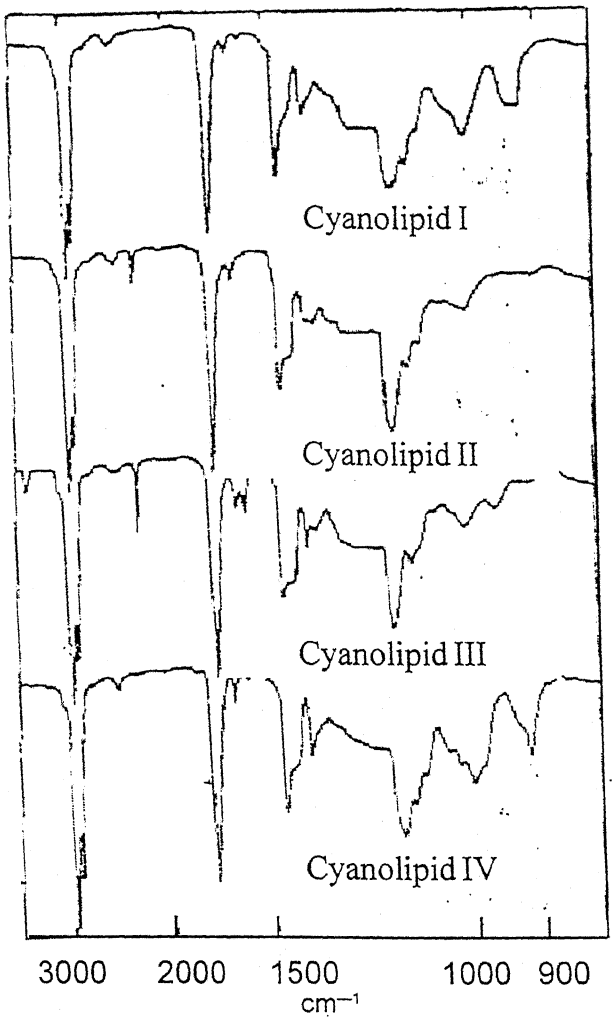


Fig. 1. Infrared spectra (1% $CHCl_3$) of purified cyanolipids

pattern displayed in Fig.2. The cyanohydrin esters (I and IV) migrate considerably further than the α, β–unsaturated nitrile esters (II and III) and indeed are less polar than ordinary triglycerides. Cyanolipid IV in *Ungnadia speciosa* seed oil, fig. 2, lane 6, is the least polar of the four. In lanes 1 and 2, analysis for *cardiospermum halicacabum* and *cordia verbenacea* seed oils, respectively, indicate that each contains a fast moving spot due to cyanolipid I, which is not quite as mobile as IV. Thus, I and IV are resolved from normal triglycerides and from each other by benzene.

Koelreuteria oil (lane 3) contains both II and III and displays an elongated spot encompassing both cyanolipids and triglycerides. Both *Nephelium* (lane 4) and *Stocksia* (lane 5) oils, which contain II and III, respectively, also give elongated spots but with some evidence of partial resolution of cyanolipids from triglycerides.

When TLC plates are developed with ether-hexane (1:3), the pattern shown in Fig. 3 is obtained. Now spots due to cyanolipids I and IV are obliterated by the triglyceride spot, but cyanolipids II and III are well resolved from each other and from the triglycerides. Lane 3, the *Koeleuteria* oil analysis, shows two large spots in addition to the triglyceride spot. The more mobile of these spots is also seen in the *Nephelium* oil analysis (lane 4) and is due to cyanolipid II. Cyanolipid III, which is responsible for the more polar spot in *Koelreuteria*, evidently is the only cyanolipid in *Stocksia* seed oil (lane 5). Analysis of *Cardiospermum* oil in this solvent system detected a small amount (6% was isolated) **[78]** of cyanolipid II. In addition, *Cordia* oil (lane 2) gave a very faint spot for II and *Ungnadia* oil (lane 6) contained traces of both II and III as shown by the dotted boundaries.

The chromatograms in Fig.2 and 3 were overloaded to detect minor components; this caused some spots to be irregularly shaped. Polar spots near the origin in Figs. 2 and 3 are at least partially due to free fatty acids. R_f values of specific components on TLC plates may vary slightly depending on the adsorbent used, the method of drying, whether or not the atmosphere of the development tank is saturated with solvent vapors and the amount of materials applied to the plate.

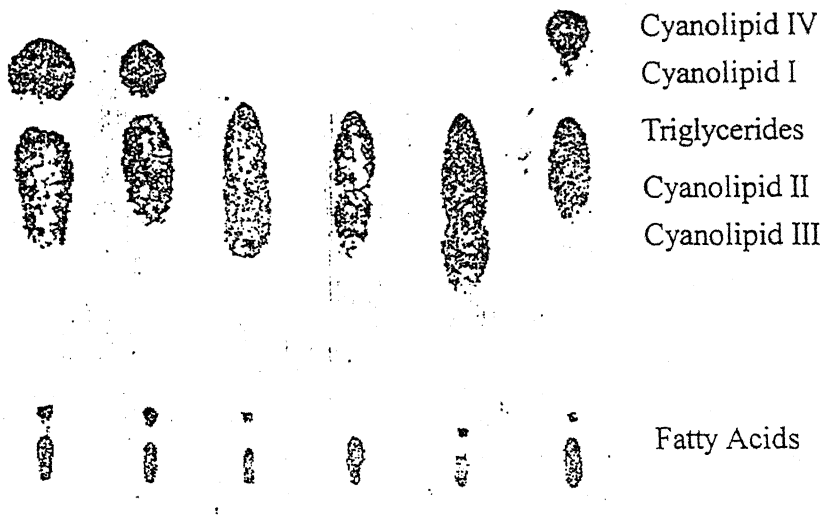


Fig. 2. Thin-layer chromatogram of cyanolipid-containing seed oils developed in benzene. lane 1, *Cardiospermum halicacabum*; lane 2, *Cordia verbenacea*; lane 3, *Koelreuteria paniculata*; lane 4, *Nephelium lappaceum*; lane 5, *Stocksia brahuica*, and lane 6, *Ungnadia speciosa*. Visualized by charring after spraying with sulfuric acid-dichromate solution.

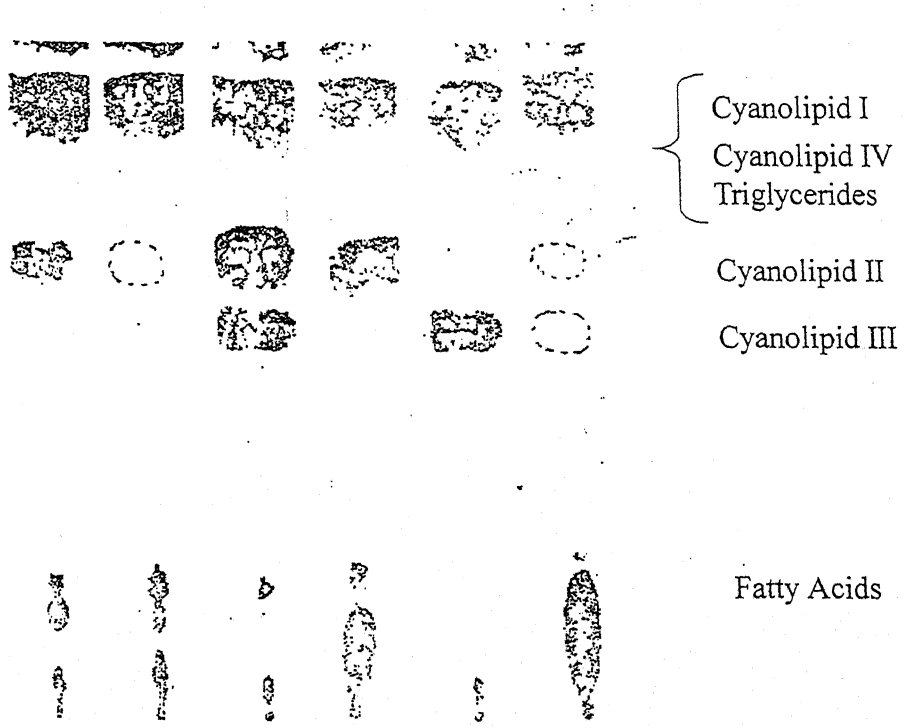


Fig. 3. Thin-layer chromatogram of cyanolipid-containing seed oils developed in ether hexane (1:3). Lane 1, *Cardiospermum halicacabum*; lane 2, *Cordia verbenacea*; lane 3, *Koelreuteria paniculata*; lane 4, *Nephelium lappaceum*; lane 5, *Stocksia brahuica*, and lane 6, *Ungnadia speciosa*. Visualized by charring after spraying with sulfuric acid-dichromate solution.

Kasbekar et al., **[89]** report analyzing kusum oil on silica gel with ether-hexane (5:95) and observing a triglyceride spot (R_f 0.55), a cyanolipid spot (R_f 0.66) and a more polar cyanolipid spot of unspecified R_f. These values imply that in this solvent system, I, which is the major cyanolipid of kusum oil **[29]** is resolved from triglycerides almost as well as with benzene (Fig. 2). The more polar spot can be assigned to cyanolipid II. This ether-hexane solvent system should also be valuable in cyanolipid detection.

Other workers, **[56, 58]** attempting the isolation of cyano materials from kusum oil, used reversed phase and argentation procedures which produced a multitude of components by resolving triglycerides (and presumably cyanolipids) according to the specificities and peculiarities of these TLC systems. These results complicated the kusum oil picture at the time because the cyanolipid components still overlapped certain triglyceride components.

4. Gas-Liquid Chromatography

GLC has been used to some extent for direct analysis **[107]** of Sapindaceae seed oils containing cyanolipids **[30, 32, 78]**. Even though GLC provided some of the first direct evidence for cyanolipids, the results were not entirely satisfactory **[78]**. Some difficulties involving apparent decomposition were encountered with cyanolipid II and also with I **[106]**. This decomposition occurred erratically and variables which may have caused it, including temperatures, types of equipment and column packings, are obscure; the problem simply has not been investigated in depth.

Recent work **[106]** indicateds that oils containing any of the four cyanolipids can be analyzed successfully under at least one set of rather ordinary experimental conditions designed for triglyceride analysis. The GLC charts (chart speed = 0.25 in/min) reproduced in Figs. 4 and 5 were obtained with a Hewlett-Packard model 5750 instrument equipped with flame ionization detectors. Helium was the carrier gas. On-column injection to a 90×0.3 cm. stainless steel column packed with non-polar, 3% OV-1 packing was used, and the column bath temperature was programmed from 160 to 400°C at 4°/min.

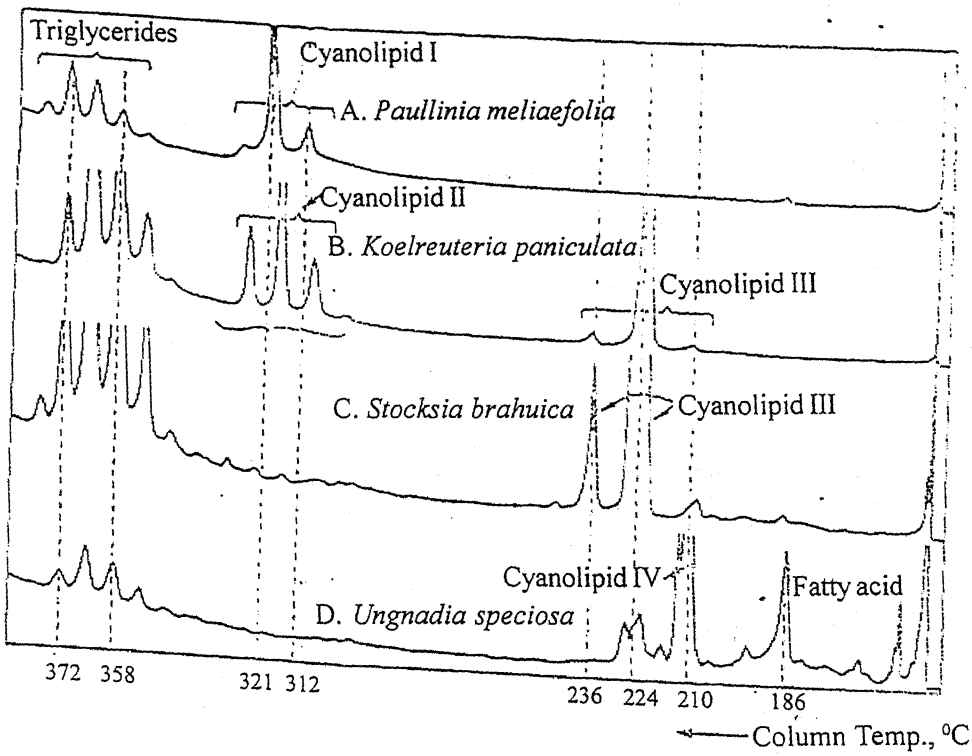


Fig. 4. Temperature-programmed GLC analysis of cyanolipid-containing seed oils. See discussion for experimental details.

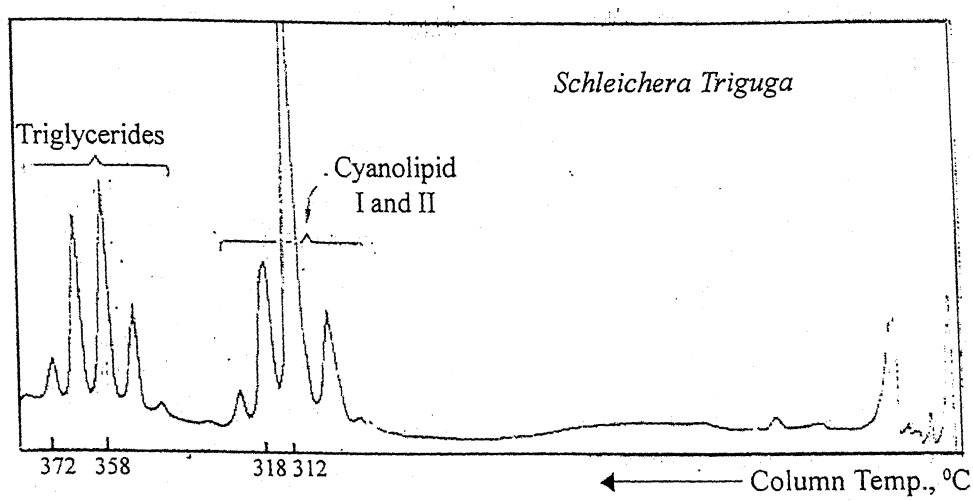


Fig. 5. Temperature-programmed GLC analysis of *Schleichera trijuga* (kusum) seed oils. See discussion for experimental details.

Figure 4A reveals that cyanolipid I, occuring in *Paullinia* oil comes off the column at a higher temperature (321°C) than any of the other three cyanolipids. Since *Paullinia* oil contains over 70% of C_{20} acids **[78]** the major cyanolipid I, peak (at 321°C) is due to a dihydroxynitrile moiety esterified with two C_{20} and residues. The two smaller peaks are due to corresponding diesters involving a C_{18} + C_{20} combination (321°C) and a C_{20} + C_{22} combination. The other type of diester, cyanolipid II, is shown as it occurs in *Koelreuteria* seed oil, Fig. 4B. Again the constituent having the dihydroxynitrile esterified with two C_{20} acyl groups presumably gives rise to the largest GLC peak observed.

Cyanolipid III emerges at a much lower temperature (224°C) than I or II primarily because it is a monoester. Figure 4C shows III alone in *Stocksia brahuica* seed oil. Since III from either source contains 90% of C_{20} acids (Table 1), the ester containing this acyl group is responsible for the largest peak.

The double peak in the GLC analysis of *Ungnadia* seed oil (Fig. 4D) is due to the presence of two monoesters; one contains a monoenoic C_{20} acid (largest peak) and the other contains a saturated C_{20} acid (smaller peak at about 212°C). At the point on the chromatogram marked 224°C, there appears to be a small amount of cyanolipid III.

Thus, it is obvious from Fig. 4 that cyanolipids III and IV are readily separable and could probably be identified by GLC, even in an oil containing both of them. However, if the major ester of cyanolipid III contained a C_{18} acyl group in-

stead of a C_{20}, it would presumably emerge at 210° C, the exact location of cyanolipid IV. In this particular case, III could not be resolved form IV by GLC.

The same sort of overlap can exist with cyanolipids I and II as demonstrated in Fig. 5. *Schleichera trijuga* (kusum) oil contains a large amount of cyanolipid I and a considerably smaller amount of II **[29]**. More importantly, both I and II in kusum oil are diesters composed primarily of C_{18}+ C_{20} acyl group combinations instead of the C_{20}+ C_{20} combinations as in *Paullinia* cyanolipid I and *Koelreuteria* cyanolipid II. The net result of this difference is that in Fig. 5 the major cyanolipid peak is at 312°C which corresponds to the elution temperature of one of the minor side peaks in *Paullinia* cyanolipid I. Slight shoulders, indicative of the resulting overlap of I and II in this oil are visible on these GLC peaks.

Although GLC data alone are insufficient for absolute identification of these cyanolipids, they provide important information that is useful in conjunction with results from other analytical techniques.

Quantitation of GLC analysis of seed oils such as these containing more than one type, or class, of material is meaningless without application of appropriate response parameters **[107]** or adjustments, hence, Figs. 4 and 5 cannot be interpreted on that basis.

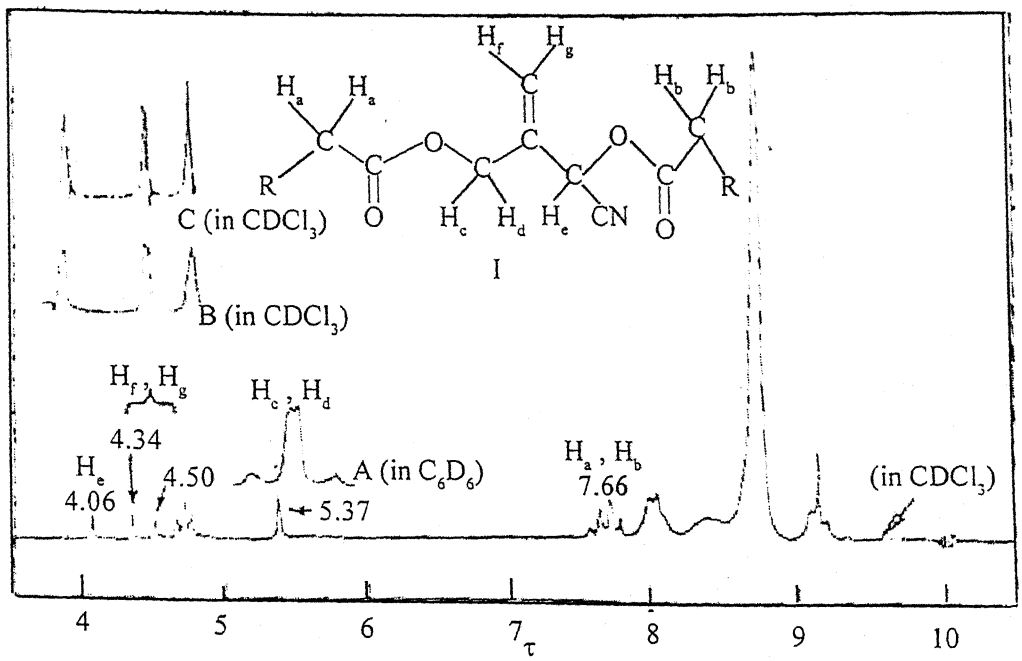


Fig. 6. NMR spectrum (100 MHz, $CDCl_3$) of cyanolipid I. Insets are intensity and scale expansions; A, in C_6D_6; B, in $CDCl_3$; and C, decoupled signals in $CDCl_3$. Taken from Seigler et al. [32]

5. Nuclear Magnetic Resonance

Pure cyanolipids : Unquestionably, the most useful and definitive tool available for the detection and structure elucidation of cyanolipids is NMR analysis. For the four cyanolipids thus far isolated, the significant signals one needs to examine are distinctive and well resolved from acyl group proton signals.

Figure 6 shows the NMR spectrum of cyanolipid I from *Cordia verbenacea* seed oil [32] . In this spectrum, signals due to protons of methylene groups α to the acyl carbonly groups (H_a, H_b) appear as two overlapping triplets, giving the observed four line pattern centered at τ7.66. The –CH_2O–protons, H_c and H_d, give a singlet at τ5.37 if analyzed in deuterochloroform; in deuteroenzene, the signal due to these same protons is two doublets, J=13 Hz (Fig. 6, inset A).

The three remaining protons of the dihydroxynitrile portion of I produce three broadened singlets at τ4.50 and 4.34 (H_f and H_g) and at τ4.06 (H_e). Inset B reveals the complexity of all three signals. Terminal methylene protons such as H_f and H_g frequently are nonequivalent and have different chemical shifts. Another peculiarity of protons of this type is that they usually have small coupling constants as shown in Fig. 6 in contrast to the 10 to 15 Hz coupling ordinarily existing between nonequivalent geminal protons. Irradiation of the H_c, H_d signal produces a partially decoupled spectrum, Fig. 6, inset C, which demonstrates that allylic coupling exists between proton H_e and protons H_f and H_g.

Cyanolipid II from *Koelreuteria paniculata* seed oil **[31]** also gives a unique NMR spectrum (Fig. 7). Protons of the two methylene groups of the dihydroxynitrile moiety of II apparently experience different shielding effects, probably caused by the cyano group being nearer one methylene group than the other; this difference manifests itself in the generation of two signals, one a singlet at τ5.13 and the other a doublet at τ5.32 for these protons. The latter signal is split by long-range coupling with the vinylic proton, H_a. The other methylene protons are not noticeably coupled with H_a by virtue of their different stereochemistry. A broad singlet, furthest downfield at τ4.45, is due to proton H_a. Portions of decoupled spectra, shown as insets A and B, are self-explanatory.

Protons of the two methylene groups adjacent to acyl moiety carbonyls of II are equivalent and yield a triplet at τ7.64 instead of the four line pattern observed for these protons in I (Fig. 6). A broadened triplet at τ4.68 due to acyl group vinyl protons completes the spectrum.

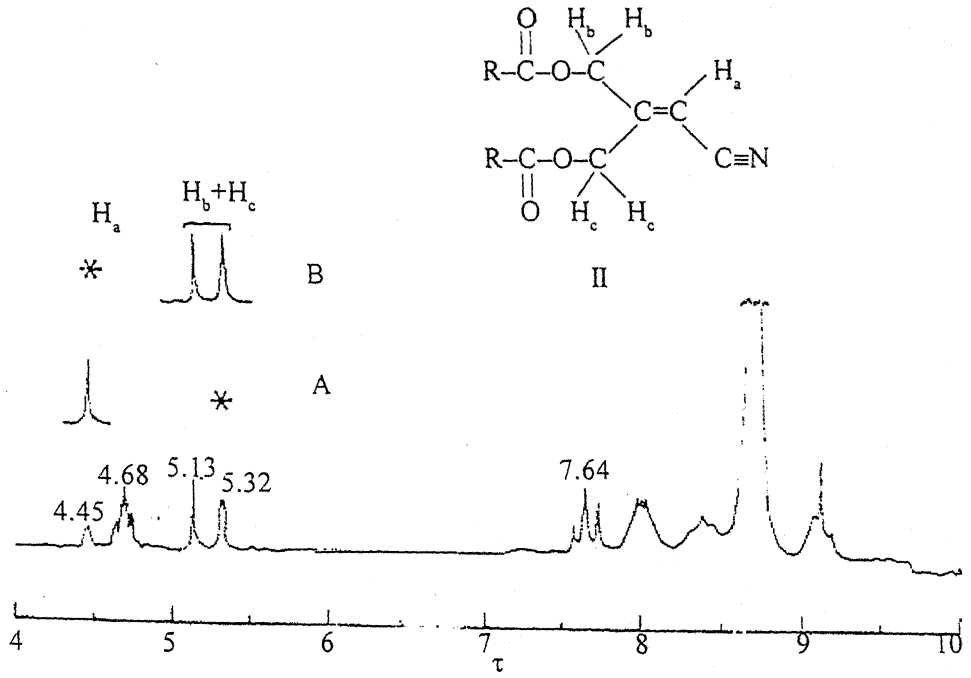


Fig. 7. NMR spectrum (100 MHz, $CDCl_3$) of cyanolipid II. Insets A and B are portions of the decoupled spectrum obtained by irradiction at points marked by asterisks. Taken from Mikolajczak et al. **[31]**

In Fig. 8, the NMR spectrum of cyanolipid III, the presence of vinylic methyl group protons is indicated by the singlet at τ8.09. The observed shoulder is due to allylic coupling (J=I-1.5 Hz) with the vinyl proton H_a. Cyanolipid III contains only one methylene group attached to oxygen; these protons (H_c) are responsible for a slighlty broadened singlet at τ5.22. Broadening of this signal is caused by weak coupling with proton H_d. As shown in Fig. 8, the H_d signal in the deuterochloroform spectrum is partially obscured by the acyl group vinyl proton signal at τ4.71. However, if deuterochloroform-deuterbenzene (9:1) **[30]** is employed as the solvent, these signals each shift in opposite directions as depicted in Fig. 9 and two well-resolved signals result at τ4.66 (acyl vinyl protons) and at τ4.90 (H_d in addition, the H_c signal shifts to τ5.26 and the vinylic methyl signal (not shown) shifts to τ8.22 when the spectrum is determined in this mixed solvent.

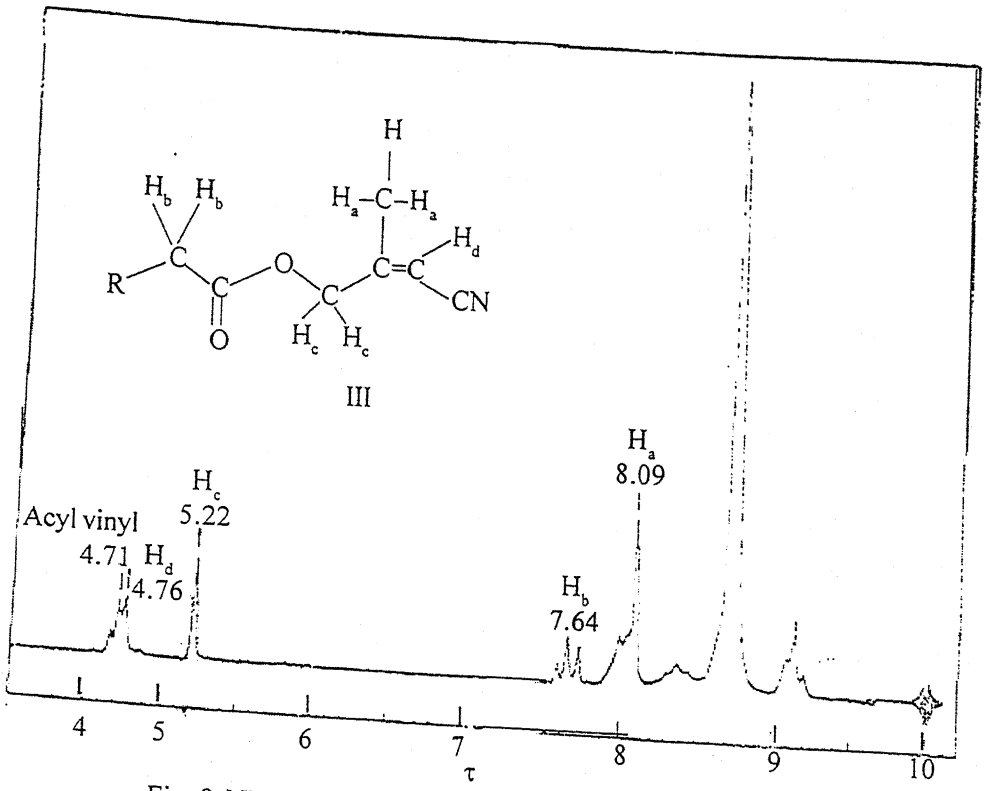


Fig. 8. NMR spectrum (100 MHz, $CDCl_3$) of cyanolipid III.

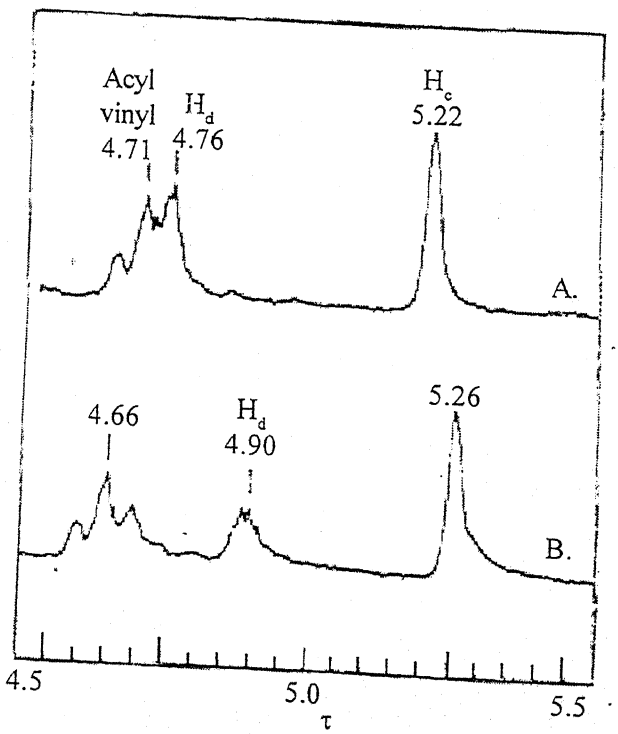


Fig. 9. Portions of intensity and scale expanded 100 MHz NMR spectra of cyanolipid III. A, in $CDCl_3$, B, in $CDCl_3$ - C_6D_6, 9 : 1. Taken in part from Mikolajczak et al. [30]

Cyanolipid IV, a monoester like III, gives the NMR spectrum displayed in Fig. 10 **[106]** which yields, in essence, the same data reported by Seigler et al., **[33]**. A vinylic methyl proton (H_a) doublet is located at τ8.14 and is coupled (J=1.5 Hz) with the terminal methylene protons. Terminal methylene protons H_d and H_c are nonequivalent and give rise to the more or less typical broadened and finely split singlets observed at τ4.83 and τ4.68; the later signal is partially hidden by the acyl group vinyl proton signal. Proton H_c, the cyanohydrin proton, gives a singlet at τ4.22 which is slightly broadened by weak coupling between H_c and at least one of the terminal methylene protons.

Cyanolipid-containing seed oils : Recently a note recommending NMR analysis of unfractionated seed oils as both a qualitative and quantitative measure of cyanolipids present was published **[84]**. This procedure is, indeed, an effective means of determining the occurrence and amounts of cyanolipids in oils; the limit of detection was not specified but is probably on the order of 1 or 2% **[94]**. Fig. 11, significant portions of the NMR spectra of all four cyanolipds and also of unfractionated *Koelreuteria* seed oil (Fig. 11 E) are vertically aligned and they show clearly the relationship of the various signals to each other.

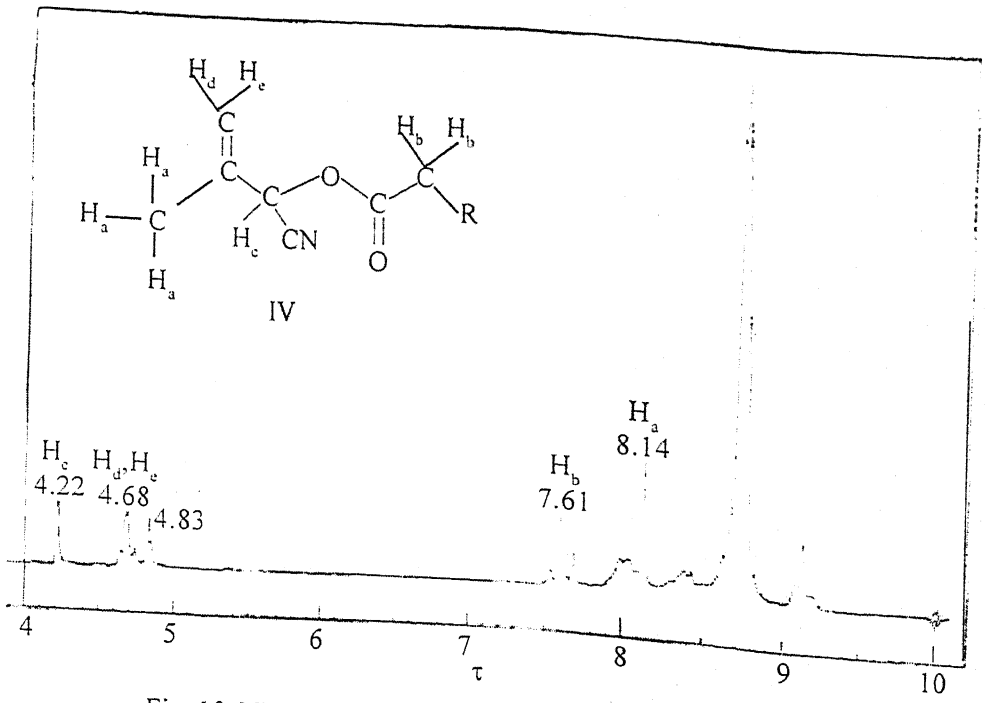


Fig. 10. NMR spectrum (100 MHz, $CDCl_3$) of cyanolipid IV.

The fact that each cyanolipid gives a distinctive NMR spectrum implies that, theoretically, one ought to be able to analyze seed oils containing any combination of cyanolipids and identify and determine the quantity of each one present. The spectrum of *Koelreuteria* oil **[106]** (Fig. 11E) exemplifies the type of NMR spectrum that results from analysis of a cyanolipid-bearing seed oil. Diagnostic signals for cyanolipids II and III. Which total about 40% of *Koelreuteria* oil, are free from interference by triglyceride signals. A similar spectrum obtained for unfractionated *Sapindus mukorossi* oil, which contains only 13% total cyanolipid (II), is also quite definitive **[106]**.

This procedure, involving NMR analysis of relatively small quantities of unfractionated seed oil, has been successfully utilized to detect and identify cyanolipids in many species of Sapindaceae **[75]**.

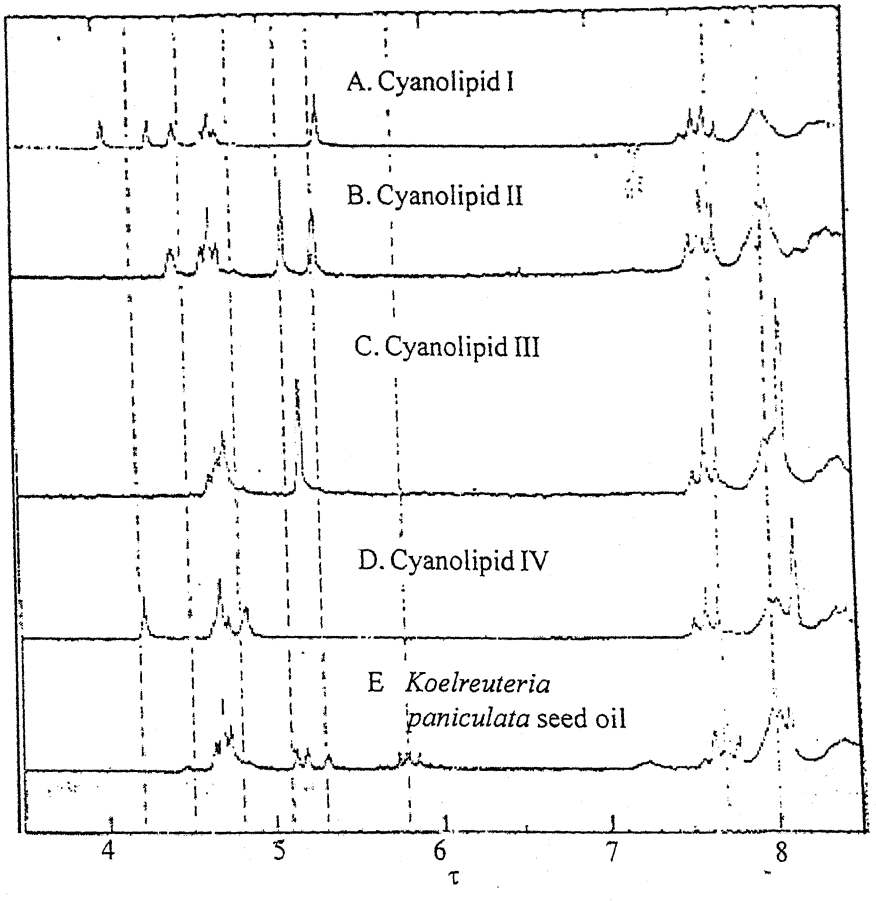


Fig. 11. Partial NMR spectrum (100 MHz, $CDCl_3$) of cyanolipid I, II, III and IV (A, B, C and D, respectively) and *Koelreuteria paniculata* seed oil (E).

As far as quantitation of this type of NMR spectra is concerned, appropriate cyanolipid signals are compared by integration to the prominent glycerol methylene proton signal centered at τ5.80 as seen in Fig. 11E. This comparison gives the relative amounts of triglycerides and of cyanolipids in the oil. Then the contribution of each cyanolipid is evaluated by comparing the integration of appropriate proton signals with the total cyanolipid integration. It is suspected that this type of quantitation may be somewhat inaccurate, especially for low percentages, because it will reflect problems associated with integration of the NMR spectrum. In spit of

some possible difficulties, however, this NMR procedure is currently the best quantitation method available.

6. Mass Spectra

After cyanolipid fractions have been isolated from the seed oil and are free of contaminants, mass spectral analysis is helpful in the structure determination because these lipids give molecular ions. Cyanolipids I and II, the diesters, give molecular ions for all possible combinations of any two acyl groups found in the cyanolipid esterified with the dihydroxynitrile of molecular weight 113 **[31, 32]**. In cyanolipid I from *Cordia* **[32, 79]** and also from *Schleichera* **[89]**, one of the most abundant molecular ions observed is m/e=671. This molecular weight corresponds to that of the diester containing a C_{20} saturated acid and a C_{18} monounsaturated (or vice versa) acid. Other significant molecular ions from other acyl group combinations occur at m/e 727 and 725, 699 and 697, and 669 in the *Cordia* **[32, 79]** spectrum.

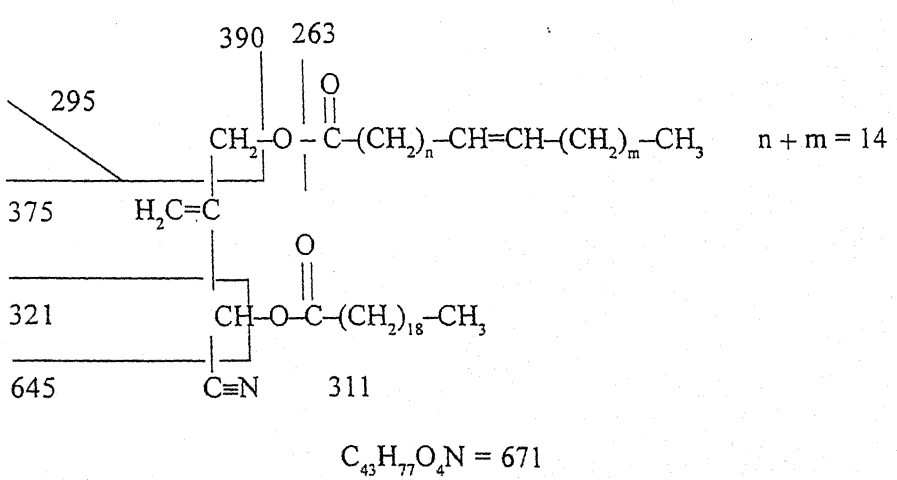


Fig. 12. Mass spectral fragmentation pattern of cyanolipid I. Taken from Kasbekar et al. **[89]**

A fragmentaion pattern (Fig. 12) was proposed for cyanolipid I from kusum oil **[89]**. Cyanolipid II encompasses a comparable range of molecular weights. In this case, the fatty acid composition of II (from *Koelreuteria* oil) shown in Table 1 indicates that the most abundant molecular ion probably would be derived from the diester incorporating a C_{20} monounsaturated acid and a C_{18} monounsaturated acid (m/e=669); the mass spectrum demonstrated that this conclusion was correct. Cyanolipids III and IV from *Stocksia* **[30]** and *Ungnadia* **[33]** oils, respectively, produce a preponderance of the molecular ion of m/e = 389 from a C_{20} monoenoic ester of the hydroxynitrile moiety.

7. Ultraviolet Spectra

Cyanolipid II from *Koelreuteria* **[31]** showed UV absorption in Cyclohexane at 208μm, ε =14,340 (assuming a mean molecular weight of 669). The corresponding monoester with an α, β-unsaturated nitrile grouping (III) from *Koelreuteria* oil also gave a maximum at 208 μm in cyclohexane, ε = 12,600 (assuming a mean molecular weight of 389) and III from *Stocksia* **[30]** gave ε = 13,070. Cyanolipids I and IV possess no ultraviolet absorbing chromophore and cannot be detected by this procedure. The absorption of small amounts of II or III in a seed oil is hidden by background absorption.

B. Isolation

1. By Preparative TLC

Even in instances where more than one type of cyanolipid occurs in a particular seed oil, each type can usually be recovered in a pure state by preparative TLC although some sacrifice of yield may have to be made. A possible exception to this generalization might surface if isolation of cyanolipids I and IV is attempted from the same seed oil under less than optimum conditions. Benzene with silica gel plates gives a partial resolution of these two components and if reasonable care is exercised in removing bands from the plate, some uncontaminated I and IV should be recovered. This problem exists with oils containing any combination of cyanolipids including I and IV. Of course, I and IV are readily recovered from oils in which they occur singly. Cyanolipids II and III, whether found in an oil singly or together, may be resolved with ether hexane (1:2) **[78]** and perhaps also with ether-hexane(5:95) **[89]**.

Thus, if I or IV (or both) are present, a separation with benzene is done ; if II or III (or both) are present, the oil must be respotted on a second plate which is developed with ether-hexane (1:2). Other uninvestigated solvents may be equally effective for achieving these separations.

In one instance **[100]** an improper solvent system was used for preparative TLC of kusum oil and resulted in an incorrect conclusion. The spot assumed to be triglyceride was isolated using ether- hexane-acetic acid (25 : 75 : 1), but the recov-

ered material liberated HCN; hence the investigators concluded that they had isolated a triglyceride-based cyanolipid. What really happened was that in this solvent system cyanolipid I of kusum oil was overrun by triglyceride components (see Fig. 3). Therefore, the positive HCN test which could not have been caused by noncyanogenic cyanolipid II, was due to a mixture of I and triglycerides.

While reported preparative TLC separations were probably achieved on hand-spread silica gel layers of 1 mm or less, modern precoated plates with 2-mm silica gel layers are equally suitable **[106]**. Loading of up to 200 mg of oil/1-mm layer (20×20 cm plates) was reported **[31, 78]** heavier loading with little or no deterioration of resolution is practical with 2-mm layer plates. However, a slight-to-moderate increase in solvent system polarity may be required with commercial precoated plates to produce migration distances comparable to those obtained on ordinary hand-spread Silica Gel G layers.

For oils that contain cyanolipids as major constituents, the TLC bands can be visualized simply by viewing the developed plate over an incandescent lamp in a darkened room **[78]**. This procedure eliminates the need for removal of fluorescent dyes from the recovered cyanolipid. *Note :* This method of visualizing TLC bands is not peculiar to cyanolipids and is probably applicable to any major constituents.

2. By Column Chromatography

Cyanolipid III is redily isolated in a pure state by column chromtography of

Stocksia seed oil **[30]** on silica gel. Triglycerides were first partially eluted with hexane and then cyanolipid III was eluted with ether hexane. Similarly, chromatography of *Ungnadia* seed oil with hexane containing a little ether **[33]** yielded pure cyanolipid IV.

However, column chromatographic techniques have generally failed in attempts to isolate cyanolipids I and II primarily because of insufficient resolving power. Seed oils to which this method has been unsuccessfully applied include kusum oil **[45, 56, 59, 89]**. *Cordia* oil **[32]** and *Koelreuteria* oil **[31]**. Cyanolipid I and II have structural features more like triglycerides than III and IV do because they are diesters; this characteristic makes resolution I and II from triglycerides by column approaches tried thus far impractical.

3. By Countercurrent Distribution

Cyanolipid I was initially isolated from *Cordia verbenacea* seed oil **[32, 78]** by countercurrent distribution in a 200-tube apparatus. The solvent system used was made up of 10 ml of upper phase and 40 ml of lower phase from an equilibrated hexane-nitroethane mixture. Transfers made after the fundamental 200 were collected in a fraction collector and materials contained in these fractions were recovered by removal of solvent. Triglyceride components were located in transfers 300–530 and cyanolipid I in transfers 450–750. These numbers mean that the cyanolipid moved slightly slower than triglycerides and that there was some overlap.

Due to the large capacity of this type of instrument (on the order of 10-50 g), this method is suitable for recovering appreciable quantities of Cyanolipid I from a seed oil. Solubility characteristics of Cyanolipids II, III and IV in countercurrent distribution. Solvent systems are unknown and attempts to fractionate oils containing any of these materials by this technique should be preceded by appropriate solubility tests. Useful separations are probably attainable with proper adjustment of variables.

4. By Distillation

Kasbeker et al., **[89]** report the recovery of a cyanolipid-rich fraction (bp 230°C) by distillation of kusum oil in a wiped film molecular still at 5×10^{-5} mm Hg. The degree of contamination of the distillate was such that further purification by preparative TLC was required. This lack of purity, the risk of thermal degradation or rearrangement and the experimental complexity of performing molecular distillations all combine to make this method a last resort.

IV. CHEMISTRY OF CYANOLIPIDS

The chemistry of cyanolipids is similar to that of glycerides or other fatty acid esters except for the troublesome instability of the hydroxy and dihydroxynitrile moieties on which they are based. While early work **[32, 79]** demonstrated that normal saponification reactions applied to these cyanolipids did not liberate glycerol, ethylene glycol or any other common polyhydric alcohol, it also recognized that befor the true identity of the alcohols present could be learned, other hydrolysis methods and perhaps derivatization would be required. Even lipase (EC 3.1.1.3) was used to hydrolyze cyanolipid III **[30]**, and hydrolysis actually occurred, but in spite of these mild conditions no hydroxynitrile was isolated.

More chemical reactions were applied to cyanolipid I than to the others because I was the first to be isolated, hence more data were essential for its unequivocal identification than were required for cyanolipids isolated later. Many unsuccessful or unproducitive reactions which had been applied to I were omitted with later isolates.

In most hydrolysis reactions designed to free the hydroxynitrile portion of the cyanolipids, it was more convenient **[30, 78]** to work with hydrogenated cyanolipids, or with partial hydrogenolysis products in the case of the diesters. Saturation of the hydroxynitrile double bond added a degree of stability to the compounds in their unesterified form, but even with this added advantage they were troublesome to manipulate.

A. Cyanolipid I

1. Hydrogenation of I

Reaction of cyanolipid I form *Cordia* seed oil [32] with hydrogen was erratic but nearly always produced two products (X, XI) as shown in Fig. 13. One (X) was simply the product of hydrogenation of the alcohol moiety double bond (which

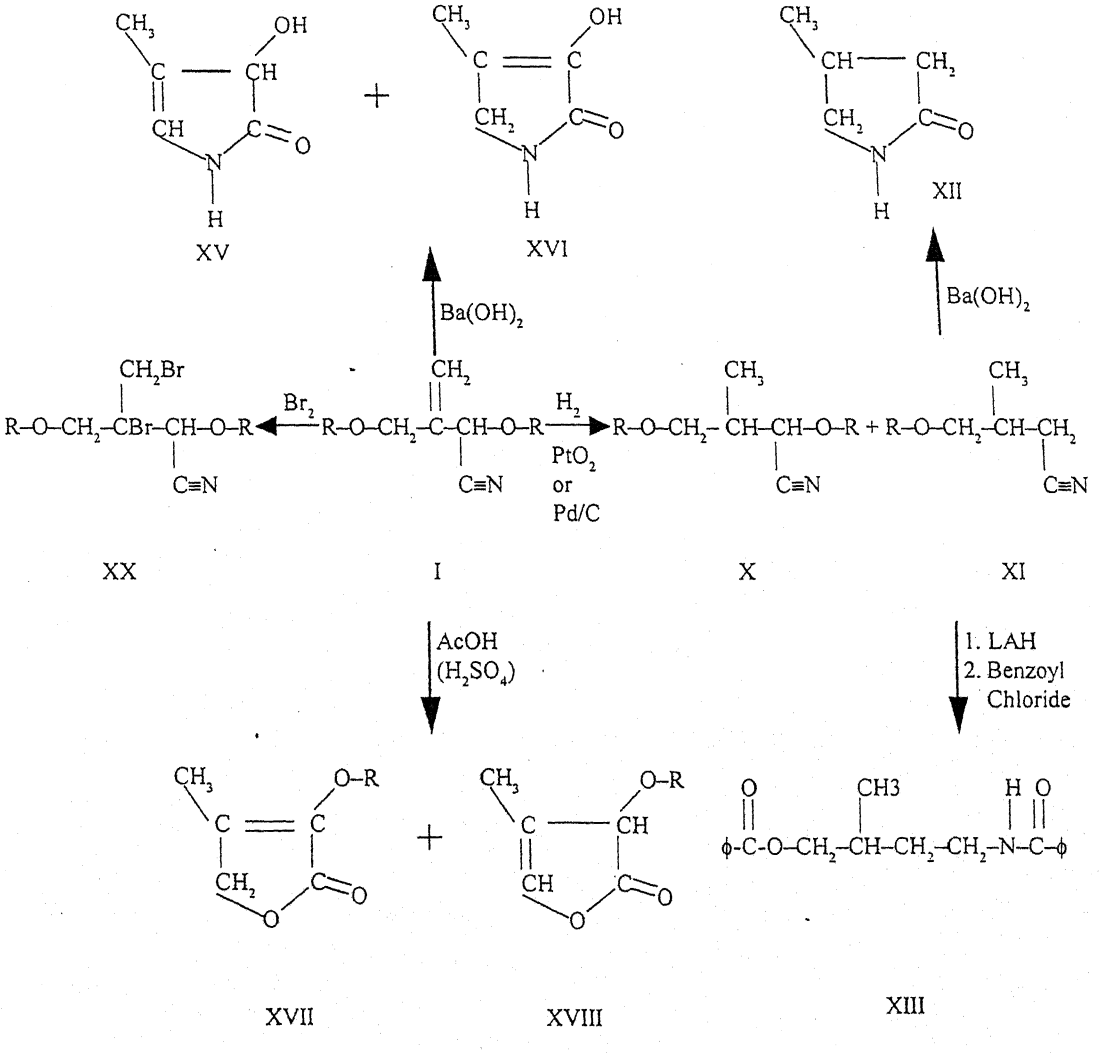


Fig. 13. Reactions of cyanolipid I and its derivatives. Taken from Seigler et al.[32]

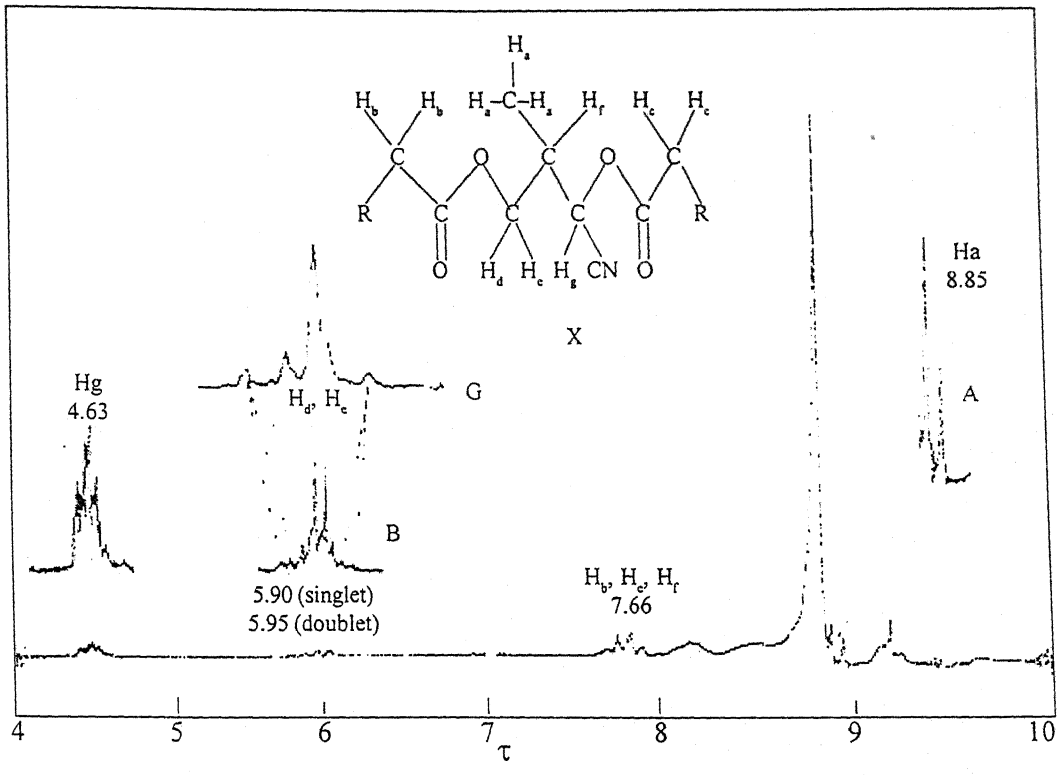


Fig. 14. NMR spectrum (100 MHz, $CDCl_3$) of hydrogenated cyanolipid I (X, Fig. 13). Insets A and B are intensity expansions; inset C is an intensity and scale expansion of the decoupled signal. Taken from Seigler et al. [32]

apparently reacts rapidly) along with partial reduction of the acyl group unsaturation. Formation of this product is favored by a short (15 min) reaction time utilizing 10% palladium on charcoal catalyst at room temperature and atmospheric pressure. A relatively large amount of catalyst is employed because some unidentified form of poisoning is a problem, and frequently a second hydrogenation with fresh catalyst was required to completely saturate the acyl group. If hydrogenation is incomplete, a band at 967cm^{-1} due to trans unsaturation formed by bond migration is observed in IR spectra of the products.

Hydrogenated cyanolipid I (X) is resolved from the hydrogenolysis product

(XI) and coporduced free fatty acids by preparative TLC using ether-hexane-acetic acid (90:110:1). Ester X is optically active (although the sign has changed), it liberates HCN, and it can be saponified or transesterified but the free dihydroxynitrile moiety is apparently too labile to be isolated.

NMR analysis of X gave the spectrum shown in Fig. 14. Protons (H_a) of the newley formed methyl group coupled with proton H_a produce the doublet at τ8.85; the H_f signal at τ7.66 is obscured by the signal of the four protons, H_b and H_c, α to acyl carboxyl groups. An interesting feature of this spectrum is the complexity of the $-OCH_2-$ signal at τ5.90 and 5.95. This complexity is the result of the methylene group being adjacent to the newly formed chiral center whcih exists as a mixture of the two possible isomers. Since the original chiral center in the molecule (the cyanohydrin carbon) is unaffected by hydrogenation, the resulting product is a mixture of two diastereomers. In one isomer H_d and H_e are equivalent but their coupling with H_f yields the sharp doublet centered at τ5.95 (see inset B, Fig, 14); however in the other isomer, H_d and H_e are nonequivalent and together with H_f produce the typical eight line AB portion of the ABX pattern at τ5.90. The cyanohydrin proton (H_g) signal at τ4.63 is hidden by signals of vinyl protons of incompletely hydrogenated acyl chains.

2. Hydrogenolysis of I

Products : Enhancement of the hydrogenolysis product (XI, Fig. 13) yield from cyanolipid I can be achieved by substituting platinum oxide (Adams) catalyst

for palladiom in the hydrogenation reaction and by increasing the reaction time. Although both ester functions are allylic to the unsaturated center, only the

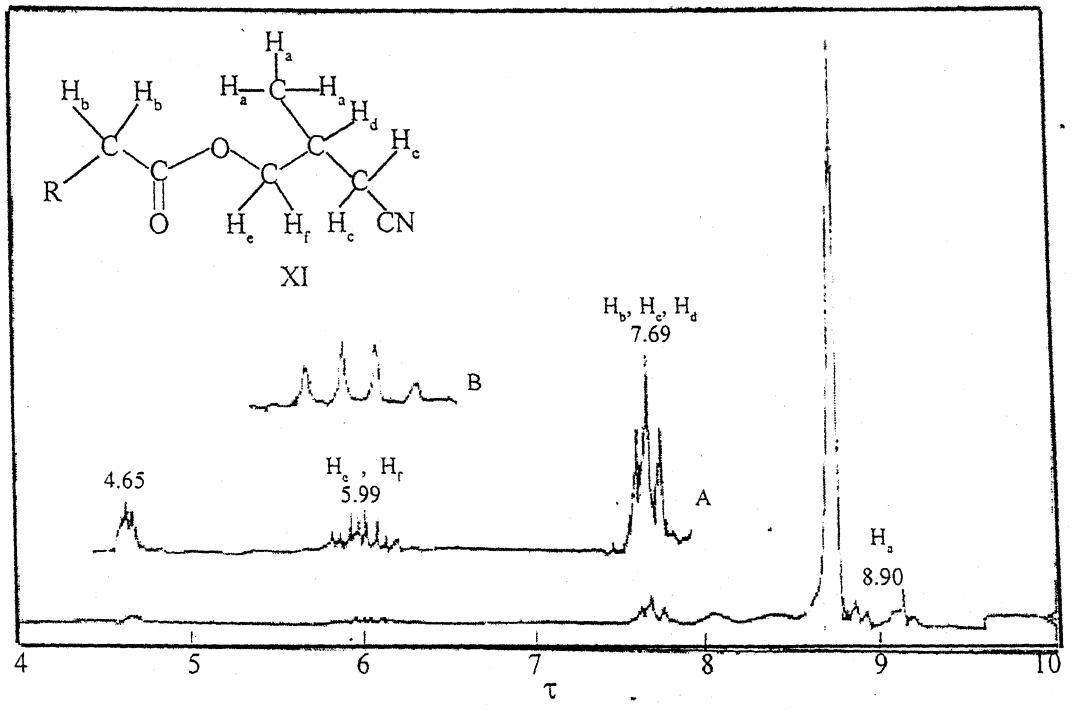


Fig. 15. NMR spectrum (100 MHz, $CDCl_3$) of hydrogenolyzed cyanolipid I (XI, Fig. 13). Inset A is an intensity expansion; inset B is an intensity and scale expansion of the decoupled signal, Taken from Seigler et al. [32]

product of hydrogenolysis at the cyanohydrin carbon was detected. This phenomenon is probably due to a combination of the inherent susceptibility of the cyanohydrin ester to hydrogenolysis and the rapid destruction of the allylic nature of the ester groupings by reduction of the double bond. The free fatty acid mixture produced by hydrogenolysis was enriched in C_{18} chain lengths compared to the corresponding acid mixture obtained by complete saponification of 1 ; this observation demonstrates that there is a slight preference (alluded to earlier) for the cyanohydrin hydroxyl of I to be esterified with C_{18} acids.

The NMR spectrum of XI is depicted in Fig. 15, Protons of the newly formed methyl group (H_a), coupled with proton H_d give a doublet at τ8.90, while the finely split H_d signal is obscured at τ7.69. Instead of two overlaping triplets due to protons of methylene groups adjacent to carbonyls as was observed in fig. 6, two superimposed triplets appear at τ7.69. These triplets are due to protons (H_c) of the new methylene group and to protons of the methylene group adjacent to the carbonyl.

Saturation of the double bond has created a new chiral center in this hydrogenolysis product (XI), and proton H_d together with protons H_e and H_f make up an ABX system. The AB portion generates the typical pattern seen at τ5.99. Decoupling by irradiation at τ7.69 causes the τ5.99 signal to collapse to a quartet as shown in inset B. Residual acyl group olefinic protons produce the multiplet at τ4.65. This product (XI) is neither cyanogenic nor optically active because both the cyanohydrin structure and the chiral center of I are disrupted by hydrogenolysis. The newly formed chiral center is, of course, a mixture of both possible optical antipodes. Compound XI is identical to the compound produced by hydrogenation of cyanolipid III.

If cyanolipid I is subjected to prolonged treatment with hydrogen in the presence of large amounts of Adams catalyst, hydrogenolysis of both acyloxy groups occurs to a small degree and yields mainly isovaleronitrile. However, lesser amounts of isoamylamine are also detected in the product.

Barium hydroxide hydrolysis of hydrogenolyzed I (XI) : Treatment of XI with saturated aqueous barium hydroxide solution in refluxing aqueous methanol, yielded γ-lactam XII as indicated in Fig. 13 **[32]**. Results of IR and NMR analysis support the indicated structure and mass spectral analysis of deuterium-exchanged XII revealed its molecular weight was 100.

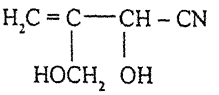
Lithium aluminum hydride reduction of hydrogenolyzed I (XI) : Ester XI, Fig. 13, gave a basic reduction product when treated with LAH in ether at room temperature **[32]**. This reduction product was converted by standard procedures to the corresponding dibenzoate shown as XIII, Fig. 13.

Because purification of the free amine was not attempted and since side reactions can occur during LAH reduction of nitriles **[108]**, it is not surprising that a crystalline benzoate was not obtained.

The NMR spectrum (100 MHz.$CDCl_3$) of crude product indicated that structure XIII. Fig. 13, was probably correct. Among the significant signals observed were a methyl proton doublet at τ8.87 and a methine proton multiplet at τ8.22 which exhibited coupling with the methyl protons. Methylene proton signals appearing at τ5.81(doublet), τ5.87 (triplet) and τ7.95 (multiplet) were assigned to the O-methylene, the N-methylene and the remaining methylene group of XIII, respectively. Decoupling by irradiation in the τ8.0 region caused the collapse of the remaining three signals described. Complex aromatic proton signals, as well as, some unassigned resonances probably caused by impurities, were also observed.

3. Barium Hydroxide Hydrolysis of I

A major barrier encountered in elucidating the structure of I was instability of the free (unesterified) dihydroxynitrile (XIV) that should result from hydrolysis



XIV

Scheme 10

of the ester. Saponification of I with sodium hydroxide in aqueous alcohol and methanolysis with sodium methoxide in methanol or with hydrochloric acid in methanol gave, in addition to the expected fatty acids or their methyl esters, intractable water soluble mixtures. Mild hydrolysis of I with barium hydroxide yielded about 25% of a mixture of cyclic amides (γ-lactams) which were provent to be XV and XVI (Fig. 13) by IR and NMR data. During the reflux period of this hydrolysis reaction, a slow liberation of HCN was detected which undoubtedly contributed to the low yield of product.

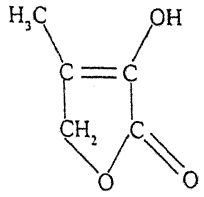
4. Acidic Hydrolysis of I

Sulfuric acid-acetic acid : By refluxing cyanolipid I with glacial acetic acid containing 1.5% of sulfuric acid as indicated in Fig. 13, it was anticipated that a stable acetate would result from hydrolysis of I and concomitant acetylation of the hydroxyl groups of diol XIV. Instead, lactones XVII and XVIII were isolated as a mixture by preparative TLC on silica gel with methanol-chloroform (4:96). The NMR spectrum (100 MHz, $CDCl_3$) of this mixture of XVII and XVIII, which differ

only in the double bond position, totally supported the indicated structures. Both contained one long chain fatty acid residue as indicated by a triplet at τ7.48 due to methylene protons α to the carbonyl group. These γ-lactones both possess vinylic methyl groups which result in two singlets at τ8.02 and 8.08; the τ8.08 signal exhibits fine splitting and is presumably attributable to XVIII. A broad two proton singlet at τ5.30 overlapped by a weaker multiplet is assigned to the ring methylene protons of XVII plus the methine proton of XVIII. Another weak multiplet partially hidden by the acyl olefinic proton signal at τ4.50 is due to the vinyl proton of XVIII.

These two unsaturated lactones are cyclization products of the γ-hydroxy acid liberated by hydrolysis of the cyano grouping and one of the two ester functions of I. The relative resistance of the cyanohydrin ester grouping to hydrolysis under these strongly acidic conditions is somewhat surprising.

Hydrochloric acid methanol : When Cyanolipid I was treated with hydrochloric acid methanol reagent, a product comprising the anticipated fatty acid methyl esters as well as a strange, unidentified material was recovered **[78]**. The new compound (XIX, 2-hydroxy-3-methylbut-2-enolactone) **[106]** was isolated by preparative TLC on silica gel with ether-benzene (5:95). By analogy with XVII and XVIII, it is apparent that this lactone arises from cyclization of the γ-hydroxy acid produced by complete hydrolysis of I.



XIX

Scheme 11

Lactone XIX was crystalline but was extermely hygroscopic and gave an irreproducible melting point in the 75-90°C range. Perhaps its most interesting property, however, was its potent odor of burnt sugar or caramel **[106]**. As the material absorbed moisture and became amorphous, this odor weakened and finally dissipated. This distinctive caramel odor was not detected with the corresponding lactones discussed above (XVII, XVIII) which differ from XIX only in having the hydroxyl group esterified. However, the comparable lactone having a 3-ethyl group instead of a methyl as in XIX has previously been shown **[109]** to possess caramel organoleptic properties and an unstable compound of uncertain structure, but isomeric with XIX, was isolated as a flavor carrier from soy sauce **[110]**.

Lactone XIX gave an IR spectrum with strong unidentified bands at 988, 1135 and 1350 cm^{-1}, a strong, rather broad ester carbonyl band at 1770 cm^{-1} with a shoulder at 1720 cm^{-1}, and a sharp hydroxyl band at 3600 cm^{-1}. The NMR spectrum of XIX showed a triplet at τ8.06 due to the methyl protons and a distorted quartet at τ5.40 due to the methylene protons; weak coupling (J=1 Hz) between them was apparent. Weak resonances from impurities presumably caused by decomposition of XIX were also present.

Sulfuric acid-dodecylbenzene suflonic acid : Kasbekar and Bringi [55] also isolated XIX, which they called β-methyl α-tetronic acid, in 4.6% yield from a Twitchell [111] process hydrolyzate of kusum oil. Again, this lactone is most probably derived from cyanolipid I since this is by far the predominant cyanolipid [29, 89] in kusum seed oil.

The Twitchell process involves [55] passing steam through a vigorously stirred mixture of oil, sulfuric acid and dodecylbenzene sulfonic acid. The lactone (XIX) was recovered by ether extraction of the lower, aqueous phase. These investigators note its hygroscopic, unstable nature but make no reference to odor.

Compound XIX, in their hands, gave a melting point of 83°C, a molecular weight of 114 by mass spectrometry and showed U.V. absorption in water at 232 μm, log ε = 3.97. The 232 μm maxium shifted to 265 μm, log ε = 3.88 when the spectrum was determined in aqueous base. Deuterated dimethylsulfoxide was used as the solvent for the 100 MHz NMR analysis of XIX [55] and a spectrum similar to that in deuterochloroform described above was obtained.

5. Other Reactions of Cyanolipid I

Bromination : Cyanolipid I from *Cordia* [32] consumed 3.1 molar equivalents of bromine to give the completely brominated product, XX, Fig. 13. The significant features of the NMR spectrum of this product are shown in Fig. 16. Creation of a new (and additional) chiral center by bromination of the terminal unsaturation of the dihydroxynitrile grouping causes the product to be a mixture of two diastereomers and thereby complicates the NMR spectrum. Protons H_a are equivalent and

give rise to two (due to diastereoisomerism) singlets at τ6.06 and 6.10. The methylene group attached to oxygen bears nonequivalent protons which are responsible for the complex overlapping pairs of doublets seen for each isomer (eight lines total) between τ5.30 and 5.70. A two-singlet signal at τ4.16 and 4.19 is due to the cyanohydrin proton, H_d.

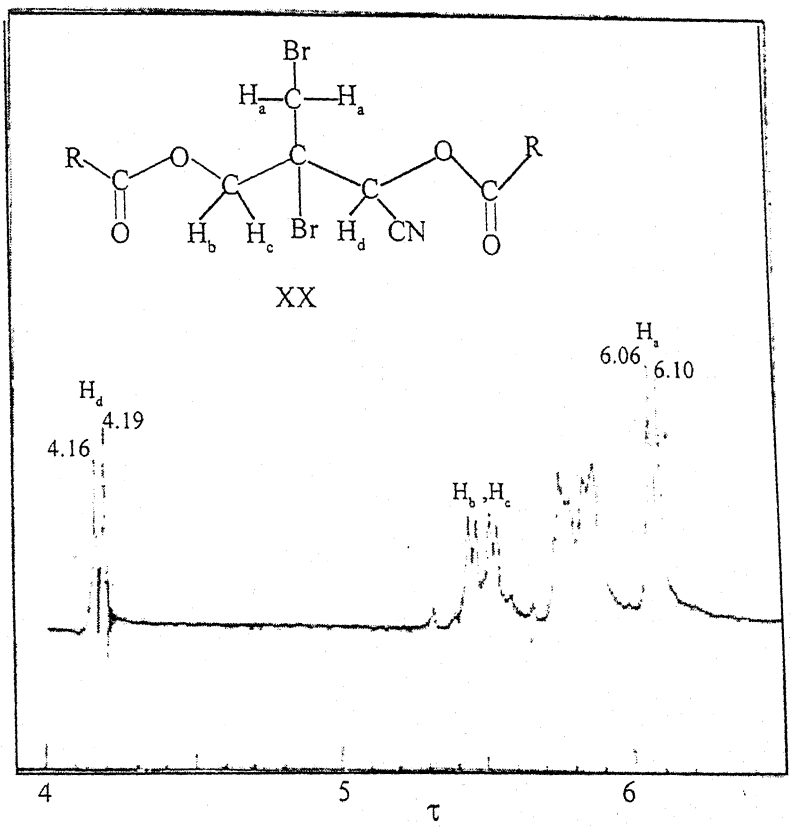


Fig. 16. Partial NMR spectrum (100 MHz, $CDCl_3$) of brominated cyanolipid I (XX, Fig. 13). Taken in part from Seigler et al. [32]

In the τ5.7 to 6.0 region of Fig. 16, a pair of distorted triplets are produced by protons on the acyl group brominated carbon atoms. The bulkiness brought about by bromine atoms on adjacent carbon atoms in the acyl groups of XX probably restricts free rotation about this carbon-carbon bond. This restriction causes the two protons on these carbons to experience different environments due to the

existence of discrete rotamers [112, 113] and hence causes these protons to have different chemical shifts as observed. Only the threo–dibromide should have been formed under these reaction conditions.

Hydrolysis of XX with acid or base failed to produce a stabile dihydroxynitrile derivative.

Periodate-permanganate oxidation : Oxidation of cyanolipid I by a modified von Rudloff procedure [114] allowed the detection of formaldehyde from cleavage of the terminal methylene. The usual solvent for this reaction, t-butyl alcohol, produced an interfering color so aqueous methanol was substituted. The reaction time was shortened to 1-5 min at pH 12, after which the pH was immediately adjusted to near 7; in this procedure the formaldehyde avoids excessive degradation which may occur under normal oxidation conditions.

Following the usaul workup, an aliquot of the product was heated with chromotropic acid reagent [115] and it gave a deep reddish-violet color which demonstrates the presence of formaldehyde. The test is conclusive evidence for the presence of terminal methylene functions in cyanolipids and is important because the weak, broad IR bands associated with this grouping are not convincing proof.

B. Cyanolipid II

1. Hydrogenation and Hydrogenolysis of II

Products : Cyanolipid II [31] responds to hydrogenation in a manner analogous to I in that hydrogen uptake is usually erratic and quantitation meaningless. It is less prone to hydrogenolysis, however, than cyanolipid I. By carrying out hydrogenations on small samples of II with 10% palladium on charcoal and a minimum volume of hexane at ambient conditions, it is possible to obtain the product of hydrogenation (XXI, Fig. 17) in about 90% purity. Once the dihydroxynitrile moiety double bond is reduced, subsequent hydrogenolysis is negligible even under

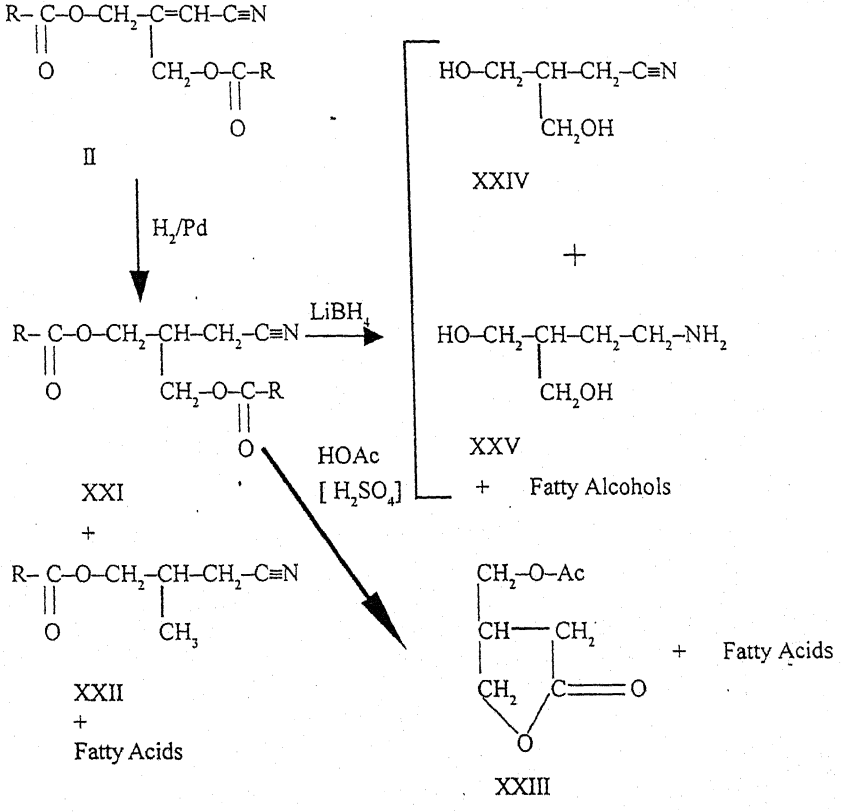


Fig. 17. Reactions of cyanolipid II and its derivatives. Taken from Mikolajczak et al. [31]

prolonged treatment in the presence of Adams catalyst. This hydrogenation procedure is important because any hydrogenolysis product XXII that is formed cannot be resolved by preparative techniques from XXI.

The hydrogenated material (XXI) gave UV and IR spectra demonstrating the virtual disappearance of absorption due to conjugation of the double bond with the nitrile grouping observed in spectra of II.

Assignment of structure XXI to hydrogenated II is valid as shown by its NMR spectrum. Since hydrogenation of II produces a racemate instead of a diastereomeric pair, there seems to be no obvious reason for the two $-OCH_2-$ groups in XXI to be different, yet they give rise to two doublets of slightly different chemical shifts, τ5.87 and 5.92 (J=2Hz). Apparently, some hindrance to free rotation exists such that one methylene group is influenced by a slightly different environment than the other. A doublet with fine splitting at τ7.57 is assigned to protons of the methylene group attached to the cyano group. The methine proton signal shows extensive coupling and is partially obscured by the τ7.57 signal and by the signal (τ7.22) due to methylene groups α to carboxyl groups.

Acidic hydrolysis of hydrogenated II (XXI) : Diester XXI, on treatment with refluxing glacial acetic acid containing 1.5% of sulfuric acid for 5 hr, afforded a good yield of lactone XXIII, Fig. 17. In contrast to lactones XVII and XVIII, Fig. 13, from cyanolipid I, XXIII has its remaining hydroxyl group acetylated. IR analysis of XXIII gave a spectrum depicting strong γ-lactone absorption (1775 cm^{-1}),

ester carbonyl absorption (1730 cm^{-1}), and typical acetate bands at 1370 and 1030 cm^{-1}.

The two methylene groups in the lactone ring of XXIII each bear nonequivalent protons which are coupled with the methine proton. This arrangement of protons is again an ABX system and results in the familiar pattern for each AB portion. One of these signals, originating with the methylene group adjacent to the carbonyl, is centered at τ7.52 (J_{AB} = 17 Hz). Protons of the other methylene produced a similar signal centered at τ5.76 (J_{AB} = 10 Hz). The third methylene group has equivalent protons whose signal is a doublet at τ5.92 (J=4.5 Hz) superimposed on the τ5.76 octet. The complex multiplet of the ring methine proton appears at τ7.2, and the presence of acetate methyl protons is confirmed by a 3-proton singlet at τ7.96.

Lithium borohydride reduction of hydrogenated II (XXI) : Reduction of fully hydrogenated cyanolipid II with lithium borohydride by standard procedures produced both dihydroxynitrile XXIV and the amino diol XXV. The meager amounts of these products available (6,8 mg) and their obvious lack of purity precluded rigorous characterization [31].

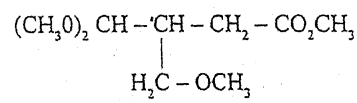
TLC analysis of XXIV with methanol-chloroform (1:3) showed that two major components of R_f 0.36 (XXIV) and 0.62 were present. The R_f 0.62 constituent was judged to be the monohydroxynitrile formed by a parallel reduction of XXII, a minor contaminant of XXI; this was substantiatad by NMR data. Other NMR and

IR data imply that structure XXIV is correct for the material having R_f 0.36.

The reported resistance of nitrile groups to lithium borohydride reduction **[116]** notwithstanding, a basic component having a strong amine odor and presumed to be grossly impure XXV was produced. Available analysis indicated that the corresponding amino alcohol from reduction of XXII was a major contaminant. Other impurities probably included XXIV and its companion nitrile from reduction of XXII.

2. Acidic Hydrolysis of II

Hopkins et al.,**[117]** first reported the isolation of an unusual material from methyl esters prepared by refluxing *Cardiospermum halicacabum* seed oil, which contains cyanolipids I and II (Table 2), with hydrochloric acid in methanol. This material was identified as methyl 4, 4-dimethoxy-3-(methoxymethyl) butyrate (XXVI), a dimethyl acetal. The IR spectrum of XXVI exhibited bands due to ether (1075-1125 cm^{-1}) and ester (1740 cm^{-1}) functions, but showed no hydroxyl or olefinic bands.



XXVI

SCHEME 12

Hopkins et al., **[117]** postulated that the precursor for this dimethyl acetal existed in the oil as part of a glyceride or glyceryl ether and was converted to XXVI by the interesterification reaction. After discovering cyanolipids I and II in

Cardiospermum oil, however, *Mikolajczak* et al., **[78]** put forth the theory that XXVI might be derived from cyanolipid II. Since cyanolipid II is a major (6%) constituent of *Cardiospermum* oil, II from *Koelreuteria* oil was treated with hydrochloric acid in methanol and the resulting product was recovered as described **[117]**. Preparative TLC with benzene recovered XXVI from this mixture in 8% yield (equivalent to Hopkins' yield) based on the weight of cyanolipid II. In deuterochloroform, he 60 MHz NMR spectrum of XXVI **[117]** shows two sharp singlets for methoxyl group protons, one at τ6.68 (3 protons) and the other at τ6.62 (6 protons). The acetal methyl protons are responsible for the 6-proton singlet. A third sharp singlet at τ6.32 is produced by the carbomethoxy protons. The $-O_2C-CH_2-CH-$ grouping gives rise to a doublet at τ7.5 partially hiding a vague multiplet, a rough doublet at τ5.63 is associated with the acetal carbon proton and an unclear signal buried under the τ6.6 signal is due to the $-OCH_2-$ grouping.

Determination of the NMR spectrum in deuterobenzene at 100 MHz as shown in Fig. 18, resulted in the acetal methyl protons exhibiting nonequivalence and appearing as two 3-proton singlets. This nonequivalence has previously been noted for sugar acetals, **[118]** in which the dimethyl acetal grouping is attached to an asymmetric center as it is in XXVI. Assignment of protons made by Hopkins et al. **[117]** is correct and agrees with the spectrum reported by Mikolajczak et al. **[78]**.

Mass spectral data as shown in Fig. 19 are also in accord with structure XXVI.

The dimethyl acetal obtained by Hopkins et al. [117] was reportadly optically active ($[\alpha]_D^{25\,C}$ +3.3, methanol); the rotation of the acetal recovered by Mikolajczak et al. [78] was not measured. However, if XXVI isolated by Hopkins et al. [117] did, indeed, originate from cyanolipid II (which is not optically active) then it would not be expected to possess activity. This discrepancy has not been resolved.

The work of Mikolajczak et al. [78] demonstrates conclusively that XXVI can be prepared from II and a plausible mechanism, shown as Fig. 20, for this

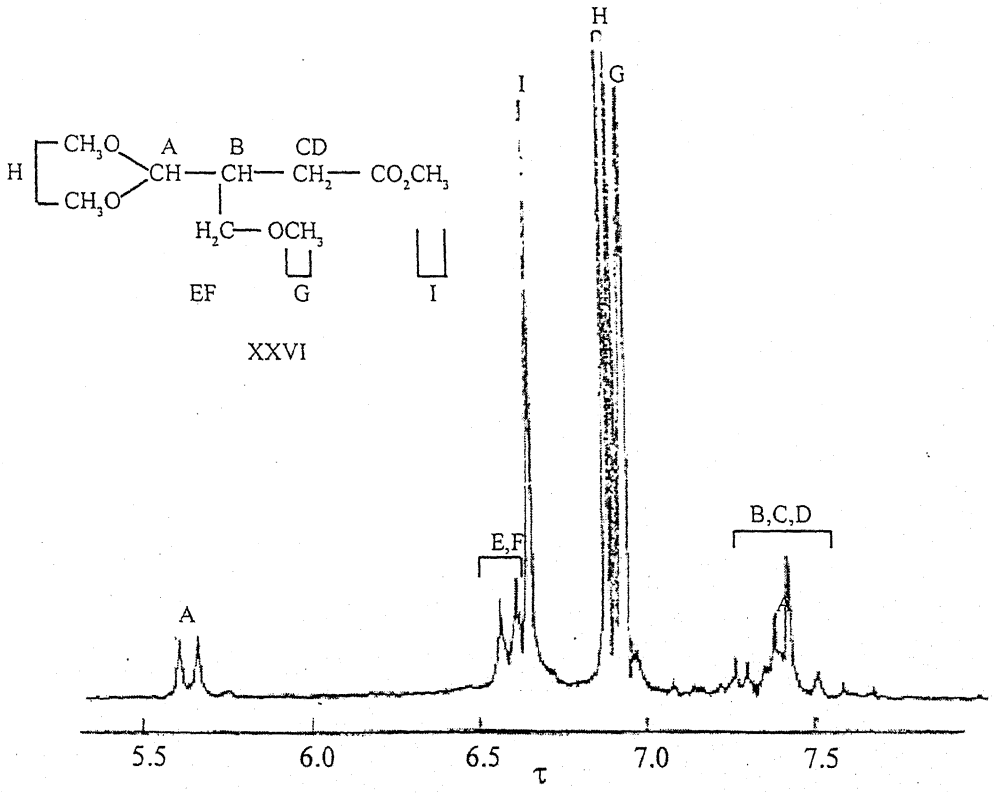


Fig. 18. Partial NMR spectrum (100 MHz, C_6D_6) of XXVI. Taken from Hopkins et al. [117]

conversion has been advanced. It includes a series of electronic shifts initiated by protonation of the nitrile group and acid-catalyzed attack of methanol on one of the allylic ester groups. The enol ester grouping thus formed could then collapse to an aldehyde which can undergo acid-catalyzed addition of methanol as shown to yield the dimethyl acetal. Conversion of the cyano function to the corresponding methyl ester under these conditions is feasible and actually does occur in formation of lactones XVII and XVIII from I as already discussed. This mechanism nicely accounts for the observed transformation of II to XXVI, but others are also possible.

It is a little surprising that lactone XIX, which should have been formed from the large amount of cyanolipid I in Cardiospermum oil by the acidic methanol treatment (discussed earlier), was not detected by Hopkins et al. [117] Perhaps water solubility or instability under distillation conditions may have precluded its detection.

3. Bromination of II

In contrast to cyanolipid I, which was completely brominated by bromine in CCl_4, cyanolipid II consumed only 2.2 molar equivalents of bromine; the dihydroxynitrile olefinic bond survived unaltered **[31]** as demonstrated by that portion of the NMR spectrum of the product presented in Fig. 21. If one compares this spectrum with that of cyanolipid II. Fig. 7, it becomes evident that the τ4.70 triplet in Fig. 7 due to acyl vinyl protons has been transformed by bromination into two triplets in Fig. 21 at τ5.76 and 5.88 due to protons on the brominated carbon atoms

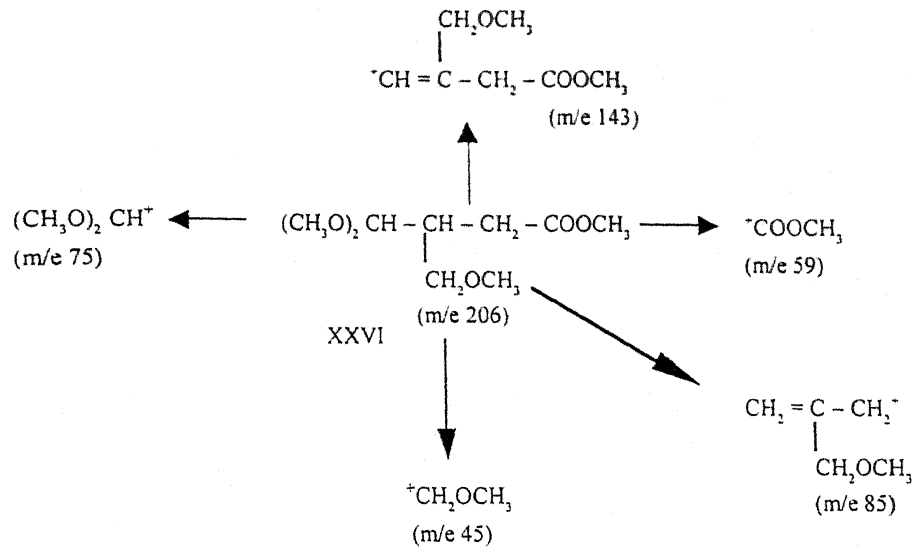


Fig. 19. Mass spectral fragmentation pattern of XXVI. Taken from Hopkins et al.[117]

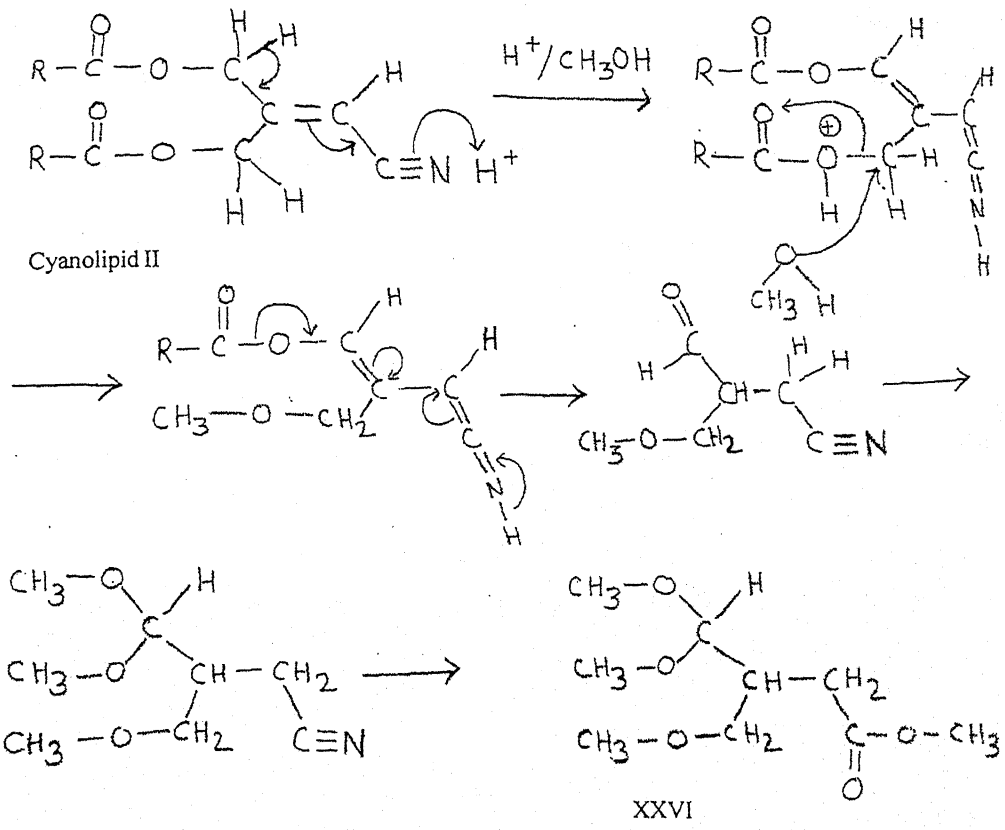


Fig. 20. A proposed mechanism for formation of XXVI. Taken from cyanolipid II . Taken from Mikolajczak et al. [78].

(see discussion about bromination of I). Signals emanating from the five protons of the dihydroxynitrile moiety are virtually identical in both spectra.

Resistance exhibited by the double bond of the dihydroxynitrile moiety of II to bromination is presumably brought about by electron-withdrawing effects of the nitrile function in conjugation with the olefinic bond. Certain compounds having similar electronic characteristics also show diminished reactively toward bromine.

C. Cyanolipid III

1. Hydrogenation of III

Products : Rapid hydrogenation of small samples of III with palladium on carbon at imbient conditions produced good yields of hydrogenated ester XXVII, Fig. 22, but some degree of hydrogenolysis always occurred **[30]**. Catalyst poisoning was again evident. The small amounts of fatty acids from hydrogenolysis were easily removed by column chromatography, leaving only XXVII. UV and IR analy-

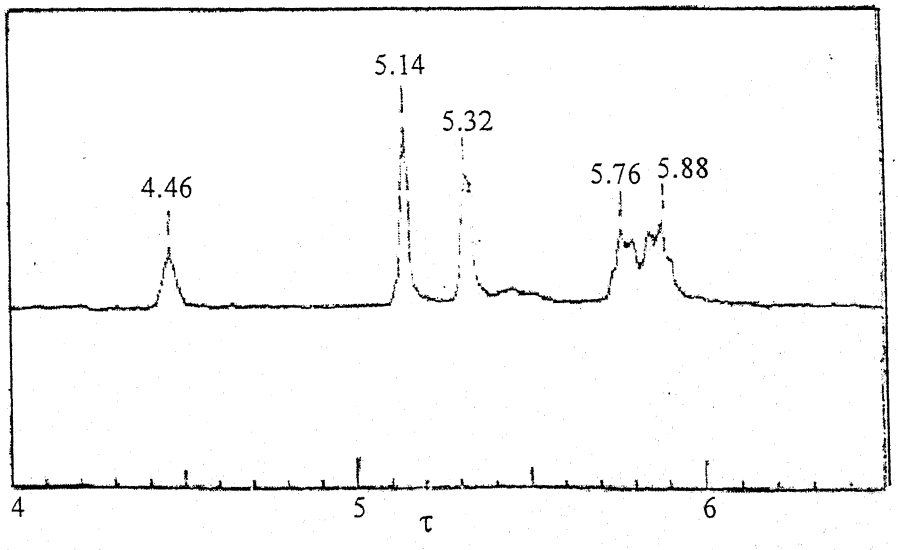


Fig. 21.. Partial NMR spectrum (100 MHz, $CDCl_3$) of brominated cyanolipid II.

sis of XXVII demonstrated that absorption due to conjugation, present in both spectra of III, was essentially eliminated. A relatively weak –C≡N band at 2240 cm^{-1} in the IR spectrum was discernible and was actually associated with XXVII, not with unreacted III. The hydrogenated material. like its precursor, III, was not cyanogenic under normal test conditions.

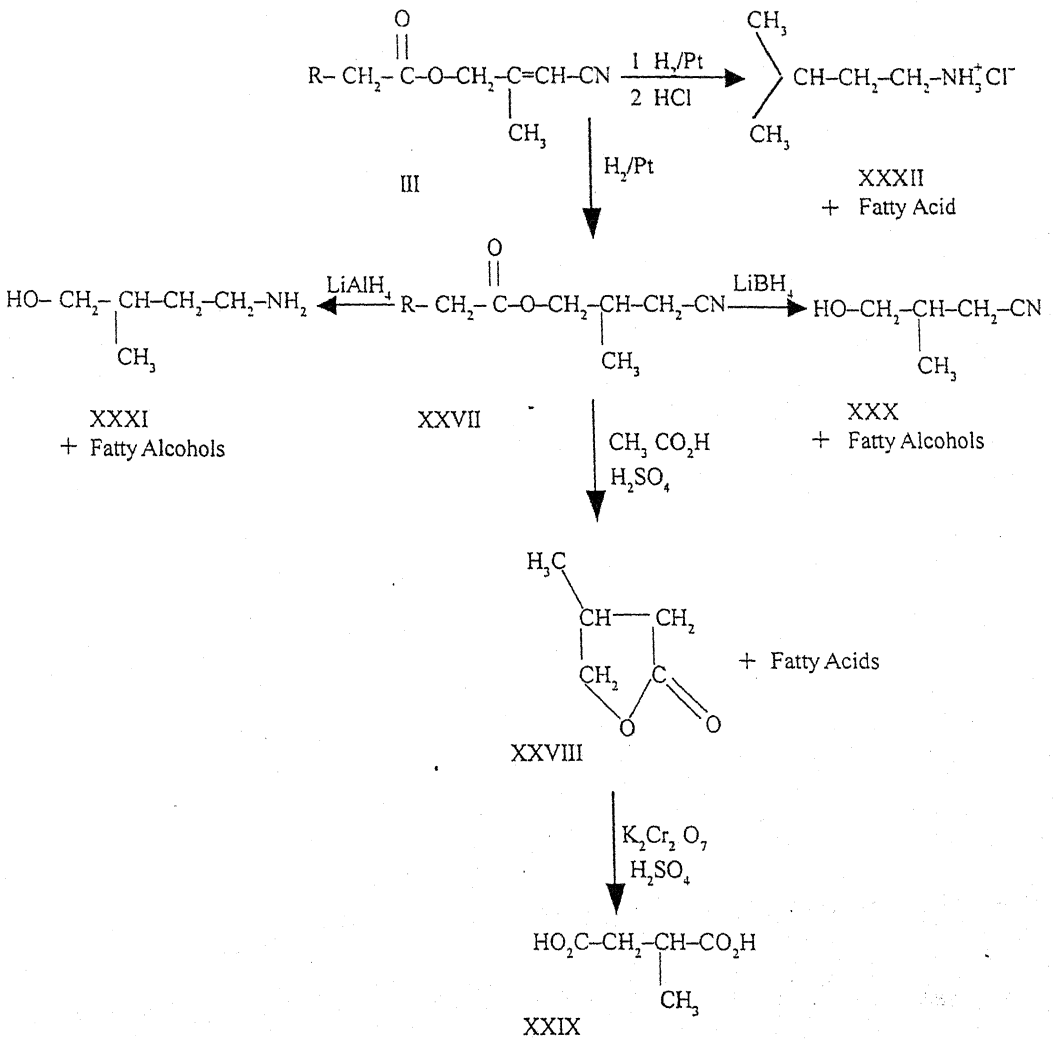


Fig. 22. Reaction of cyanolipid III and its derivatives. Taken from Mikolajczak et al. **[30]**

NMR analysis of XXVII gave a spectrum identical with that shown as Fig. 15, thereby verifying that the hydrogenation product of cyanolipid III is the same compound as the partial hydrogenolysis product (XI) from cyanolipid I.

Acidic hydrolysis of hydrogenated III (XXVII) : Reaction of XXVII (Fig. 22) with a glacial acetic acid-sulfuric acid mixture provided product XXVIII in good yield **[30]**. The IR spectrum of XXVIII showed a conspicuous γ-lactone band at 1770 cm^{-1}, and TLC analysis demonstrated that two contaminants were present in insignificant amounts.

The NMR characteristics of XXVIII were similar to those of lactone XXIII derived from hydrogenated cyanolipid II except for a methyl proton doublet at τ8.85 (J=6 Hz). Nonequivalent protons of the methylene group adjacent to the carbonyl and the methine proton both give rise to overlapping signals at τ7.59. Protons of the methylene group bearing the ring oxygen coupled with the methine proton produce the AB portion of an ABX pattern at τ5.90.

Additional evidence verifying structure XXVIII was provided by oxidizing the lactone to methyl succinic acid, XXIX, Fig. 22, with potassium dichromate in aqueous sulfuric acid. Identification of XXIX by melting point, NMR and mass spectral data was unequivocal **[30]**.

Lithium borohydride reduction of hydrogenated III (XXVII) : Lithium borohydride reduction **[30]** of hydrogenated III (XXVII) gave only nitrile XXX, Fig. 22, in about 35% yield; no amino alcohol was detected. The IR spectrum of

XXX displayed prominent hydroxyl (3690 cm^{-1}) and moderate nitrile (2255 cm^{-1}) absorption.

Protons of methylene groups adjacent to both the oxygen and the cyano group of XXX are AB portions of ABX systems and give rise to eight line NMR signals at τ6.52 and τ7.60, respectively. A methine proton multiplet (τ7.98) and a methyl proton doublet (τ8.94) were also observed. Finally, a singlet due to a deuterium-exchangeable proton (presumably on the hydroxyl) was present at τ8.58.

Lithium aluminium hydride reduction of hydrogenated III (XXVII) : As experienced in the corresponding reaction with XI, Fig. 13, problems were encountered in isolating a product of acceptable purity from the LAH reduction of XXVII. These difficulties were due mainly to side reactions and to the small quantities of material available. Amino alcohol XXXI was tentatively identified **[30]** by NMR data but details were not reported.

2. Hydrogenolysis of III

It was mentioned earlier that hydrogenation and partial hydrogenolysis products from cyanolipids I and II, specifically X, XI, XXI and XXII, do not appreciably undergo further hydrogenolysis except under forcing conditions. Conditions found to be conducive to increased production of hydrogenolysis product from III were the use of exorbitant quantities of Adams catalyst (60 mg per 100 mg of III), and allowing the reaction to proceed for 4-5 hr. It was discovered that this treatment also reduced the nitrile group yielding a saturated alkyl amine as shown in Fig. 22.

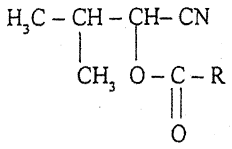
Therefore, recovery procedures designed to trap this volatile, water-soluble amine were implemented [30] and it was isolated as the hydrochloride XXXII. After thorough drying, it melted at 215-218^{0}C and no depression was observed on admixture with authentic isoamylamine hydrochloride.

NMR analysis of hydrochloride XXXII as a D_2O solution gave a rather simple, clear-cut spectrum. A doublet at τ9.09 (J = 6 Hz) was the isopronyl methyl proton signal, and a complex multiplet centered at τ8.45 was assigned to the methine proton plus the proton of the methylene group bearing the isopropyl function. Finally a triplet at τ7.01 (J= 7 Hz) originated from protons of the methylene group attached to nitrogen. A corresponding NMR spectrum of authentic isoamylamine hydrochloride was identical to that described for XXXII.

D. Cyanolipid IV

1. Hydrogenation and Hydrogenolysis of IV

Treatment of IV with hydrogen in the presence of palladium on carbon [33], provided the saturated product XXXIII, which gave $[\alpha]_D^{25}$ = +27.90 (C=0.43, in hexane). The NMR spectrum of this saturated ester in $CDCl_3$ exhibited two overlapping doublets at τ8.78 (J= 6 Hz) caused by the two methyl groups and a doublet (J= 5.6 Hz) at τ4.82 due to the proton on the cyanohydrin carbon. The signal of the isopropyl methine proton was undoubtedly obliterated by signals of the acyl group protons. The mass spectrum of XXXIII gave a molecular ion of m/e = 393 which is in accord, with the value anticipated by extrapolation of mass spectral data for IV, the unsaturated compound.



XXXIII

SCHEME 13

The statement is made **[33]** that some hydrogenolysis always occurs with cyanolipids, but no data as to how this reaction applies to IV are reported. The hydrogenolysis product would be either isovaleronitrile as from I, or isoamylamine as from III, or a mixture of both.

Any hydrolysis procedure attempted with cyanolipid IV yielded only decomposition products from the hydroxynitrile moiety **[33]**. This result is in harmony with those obtained for the other cyanolipids and with the cyanohydrin nature of IV.

PRESENT WORK

EXPERIMENTAL

Spectrometry

Infrared (IR) spectra were determined with a Perkin-Elmer model 137 spectrophotometer on 1% solution in $CHCl_3$ or CCl_4. Nuclear magnetic resonance (NMR) spectra were obtained with a Varian HA-100 spectrometer; the solvent used were $CDCl_3$ or a mixture of $CDCl_3$ and C_6D_6. Chemical shifts were measured from internal tetramethylsilane (TMS) = τ10.0. A Beckman DK-2A spectrophotometer was used to determine the ultraviolet (UV) spectra.

Oil Recovery and Methyl Ester Formation

Oils were recovered from finely ground seeds by a 12-hr extraction with petroleum ether (bp. 40-60^{0}C) in a soxhlet apparatus. Methyl esters were prepared from the oil and from Nitrogen containing lipid fraction (NCLF) by refluxing them for 3 hr with 3% H_2SO_4 in methanol. The esters were recovered by ether extraction.

Four types of cyanolipids, present individually or in pairs have been identified in the seed lipids which are cyanogenic non-glycerol esters and are derivatives of five carbon mono or dihydroxynitrile moiety esterified with long chain fatty acid (I-IV). Out of these, one class of component is a mixture of diesters containing two fatty acid moieties esterified with 1–cyano–2–hydroxy.

Gas liquid and thin layer chromatography

GLC analysis of methyl esters were performed essentially as described by Miwa and Co-workers **[119]**. Direct GLC of triglycosides and NCLF was achieved

with an F & M model 5750 chromatographed equipped with hydrogen flame detectors.

Analytical thin layer chromatography (TLC) was on 0.25 mm layer of silica gel G. The developing solvent varied and will be described below. Spots were detected by charring the plates after they had been sprayed with a standard solution of CrO_3 in 50% aqueous H_2SO_4. Preparative TLC involved 1–mm Silica gel layer developed with an appropriate solvent system and visualization was with the fluorescent dye. Desired bands were recovered from the silica by standard procedures.

Liberation and detection of HCN

Two tests were used to detect HCN derived from various materials. One of these picrate test, depending on the reaction of HCN with alkaline picrate solution to produce isopurpuric acid **[98]**. About 75–100 mg of lipid material was placed in a test tube with 1 ml of dilute NaOH or H_2SO_4. A slip of filter paper dropped in an alkaline solution of sodium picrate (05%) was partially dried and suspended over mixture in an stoppered test tube. Test tube and contains were warned at 35–50°C for 0.5–1 hr. A positive test involves a color change of filter paper form yellow to brick red **[120]**.

The second test involved formation of Prussian blue **[99]** and was carried out as described by Seigler et al **[97]**.

RESULTS AND DISCUSSION

In present investigation we have isolated and characterized this new class of lipid in *Sapindus Obavatus* seed oil. *S. Obavatus* was found to contain cyanolipid i.e. a fatty acid diester of 1–cyano–2–hydroxymethylporp–1–ene–3–ol (fig. II).

On sigel TLC the oil of *S. Obavatus* gave two spots (triglyceride, R_f 0.86 and cyanolipid, R_f 0.60) with ether–hexane (1:3) and only a single spot with benzene.

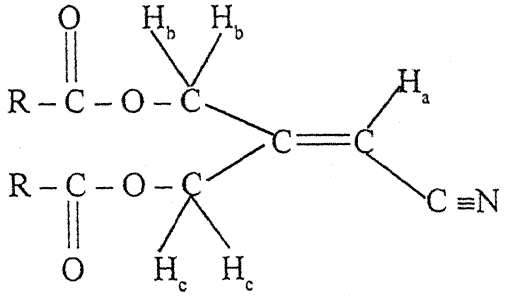
Nitrogen containing lipid fractions (NCLF) were separated from triglyceride fractions in a pure state by preparative TLC. For NCLF the plate was developed with ether – hexane (1:2).

Analysis of NCLF (II)

The IR (1% solution in CS_2) analysis of NCLF (II) revealed a band of medium intensity of 2200 cm^{-1} (–C≡N) and the IR spectra was superimposible on the spectrum of corresponding cyanolipid isolated form *S. emarginatus* seed oil.

The NMR spectra of the cyanolipid revealed proton counts, chemical shifts and multiplicities identical with those displayed by the reference sample. The NMR exhibited signals characteristic for long chain lipid group τ9.12 (rough t, 6H, terminal methyl) 8.75 (br s, shielded methylene), 7.97 –8.05 (m, protons to the double band) 7.67 (t, protons to the carbonyl function) and 4.7 (rough t, olefinic protons). The two of methylene protone H_b and H_c (II) which are adjacent to the oxygen atoms of the dihydroxynitrile moiety gave the signals at 5.3 (singlet) and 5.33

(doublet). This difference in shielding and splitting is caused by the stereochemsitry of the methylene groups; one of them is cis to nitrile grouping and other is trans. As



a result of the stereo-chemical difference between the two methylene groups, the protons of one group couple more strongly with vinyl proton than to the protons of the other methylene groups. The cyanohydrin proton (H_a) appeared as a slightly broadened signals at τ4.45.

The comparative TLC and IR characteristice coupled with NMR data established that the cyanolipid present in the is a fatty acid diester of 1–cyano–2–hydroxy–methylprop–1–ene–3–ol identical to the NCLF of *S. obavatus*. Methyl esters of all the triglycosides and accompanying NCLF had the composition as shown in Table.

Table–1, Fatty acid content of cyanolipids and triglycerides

Species	Lipid fraction	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0
S. obavatus	Triglyceride	10.3	2.6	51.8	7.4	2.2	16.2	9.5	Tr
	Cyanolipid	5.4	1.7	49.6	1.6	1.2	28.4	12.1	Tr

Only a meager amount of research has thus far been reported concerning how these strange cyanolipids are produced in plants. Mikolajczak et al [97]. First pointed out that the structures of the hydroxynitrile portions of cyanolipids I–IV suggested that they might be deried from leucine. Two of these (II & III) which occur in the seed of *koelreuteria paniculata*, have recently been shown to be derived from Leucine [78]. The aglycones of several cyanogenic glycoside have been demonstrated to come from amino acids.

Because of its basically isoprenoid structure, the dihydroxynitrile moiety of (I) has many biogenetic possibilities, it may be related, perhaps somewhat remotely, to biological compounds such as cordycepose [121] or mevaldic acid [122]. However, rather extensive studies made on the biosynthesis of other cyanogenetic materials indicate that most of them are derived from amino acids or their precursors [123–124].

REFERENCES

[1] H. Budzikiewick, J. M. Wilson and C. Djerassi, J. Am. Chem. Soc., 85, 3688 (1963).

[2] A. Ulubelen and K. Ayanoglu, Phytochemistry, 15, 309 (1976).

[3] H. Buddeck, M. H. A. Elgamol, G. S. Ricca, B. Danielli and G. Palmisano, Org. Magn. Reson., 1978, 11, 130; T. K. Chen, D. C. Ales, N. C. Baewziger and D. F. Wiemer, J. Org. Chem., 1983, 48, 3525; G. S. Ricca, B. Danielli, H. Duddeck and M. H. A. Elgamol, Org. Magn. Reson., 11, 163 (1978).

[4] K. R. Kirtikar and B. D. Basu, "Indian medicinal plants", Bishen Singh Mahendra Pal Singh, Dehradun, Vol. II, p. 830 (1984).

[5] P. K. Warrier, V. P. K. Nambiar and C. Ramakutty, "Indian medicinal plants", Orient Longman Ltd., Madras, Vol. IV, p. 339 (1995).

[6] V. S. Parmar, J. S. Rathore Rajni Jain, D. A. Henderson and J. F. Malone, Phytochemistry, 28, 591(1989).

[7] S. B. Malik, P. Sharma and T. R. Seshadri, Indian J. Chem, Sect., B, 15, 536 (1977).

[8] S. K. Talapatra, A. K. Malik and B. Talapatra, Phytochemistry, 19, 1199 (1980).

[9] K. Subramanyam, J. M. Rao and K. V. Rao, Indian J. Chem., Sect. B, 15, 12 (1977).

[10] V. P. Pathak, T. R. Saini and R. N. Khanna, Planta Medica, 49, 61 (1983).

[11] J. B. Harbone and T. J. Mabry, "The flavonoids-advances in research", Chapman and Hall, London, p. 245 (1982).

[12] T. J. Mabry, K. R. Markham and M. B. Thomas, "The systematic identification of flavonoids", Springer Verlag, New York (1970).

[13] F. Imperato, Chem. Ind., 525 (1979).

[14] T. Murakam and T. Tanaka, "Occurrence, structure and taxonomic implications of fern constituents", Springer Verlag, New York (1988).

[15] T. Murakam, N. Tanaka, H. Wada, Y. Saiki and C. M. Chem. J. Pharm. Soc. Jpn., 106 (1986).

[16] J. W. Wallace and K. R. Markham, Amer. J. Bot., 85, 965 (1978).

[17] R. N. Chopra, S. L. Nayar and I. C. Chopra, "Glossary of indian medicinal plants", C.S.I.R. Publication, New Delhi, p. 94 (1996).

[18] K. R. Kirtikar and B. D. Basu, "Indian medicinal plants", 2nd. ed., Lalit Mohan Basu and Co., Allahabad, 1, 758 (1945).

[19] "The wealth of India, A dictionary of raw materials and dustrial products", C.S.I.R. Publication, New Delhi, III, 41 (1952).

[20] J. Shinoda, J. Pharm. Soc. Jpn., 48, 214 (1928).

[21] T. J. Mabry, K. R. Markham and M. B. Thomas, "The systematic identification of flavonoids", Springer, New York, p.138 (1970).

[22] D. K. Bhardwaj, A. K. Gupta, R. K. Jain and G. C. Sharma, J. Nat. Prod., 44, 656 (1981).

[23] M. Mizuno, M. Linuma, T. Tanaka, N. Sakakibara, H. Murata and F. A. Lang, Phytochemistry, 29, 1277 (1990).

[24] M. Linuma, M. Ohyama, T. Tanaka, M-Mizuno and F. A. Lang, J. Nat. Prod., 54, 1144 (1994).

[25] S. Hakomoni, J. Biochem., 66, 205 (1964).

[26] F. Petek, Bull, Soc. Chim. Fr., 263 (1965).

[27] L. Rosenthalor, Schweiz, Apoth. Ztg. 58, 17 (1920); Chem. Abstr., 14, 556 (1920).

[28] N. N. Sengupta., J. Soc. Chem. Ind. 39, 88 (1920); Chem. Abstr., 14, 2011 (1920).

[29] K. L. Mikolajczak and C. R. Smith, Jr. Lipids 6, 349 (1971).

[30] K. L. Mikolajczak, C. R. Smith, and L. W. Tjarks, Jr. Lipids 5, 672 (1970).

[31] K. L. Mikolayczak, Smith, C. R. and Tjarks, Jr. Biochem. Biophys, Acta., 210, 306 (1970).

[32] D. S. Seigler, K. L. Mikolajczak, C. R. Smith, and I. A. Wolff, Chem. Phys. Jr. Lipids, 4, 147 (1970).

[33] D. S. Seigler, F. Seaman and T. J. Mabry, Phytochemistry, 10, 485 (1971).

[34] C. Hitchcock, in Recent Advances in the Chemistry and Biochemistry of plant Lipids, P. 4. (T. Galliard and E. I. Mercer, eds.) Academic Press, London (1975).

[35] C. Hitchcock, and B. W. Nichols, Plant Lipid Biochemistry pp. 50, Academic Press, London (1971).

[36] L. D. Bergelson, in Progress in the Chemistry of Fats and other Lipids, Vol. 10, part 3, p. 241, (R.T. Holman, ed.) Pergamon, Oxford (1969).

[37] E. E. Conn., in Toxicants Occurring Naturally in Foods, 2nd Ed., p. 299. Commitee on food protection, National Research Council, National Academy of Sciences., D. C. Washington (1973).

[38] J. M. Kingsbury, Poisonous Plants of the U.S. and Canada. Prentice Hall, Englewood, N. J. cliffs (1964).

[39] R. Hegnauer, Pharm. Zentralhalle, 99, 322 (1960); Chem. Abstr., 55, 900 d (1961).

[40] D. S. Seigler, Phytochemistry 14, 9 (1975).

[41] R. Paris, in Chemical Plant Taxonomy (I. Swain ed.) p. 346, Academic Press, New York (1970).

[42] E. W. Eckey, Vegetable Fats and Oils, p. 624, Reinhold, New York (1954).

[43] R. L. Datta, T. Basu and P. K. Ghose, Perfum. Essent. Oil Record 23, 427 (1932), Chem. Abstr., 27, 3840^{6} (1933).

[44] M. H. Coleman, J. Am. Oil Chem. Soc. 42, 751 (1965).

[45] D. N. Dhar, Fette Seifen Anstrichm. 70, 942 (1968).

[46] D. R. Dhingra, T. P. Hilditch and J. R. Vickery, J. Soc. Chem. Ind. 48, 281 T (1929), Chem. Abstr., 23, 5601^{7} (1929).

[47] T. P. Hilditch, The Chemical Constitution of Natural Fats, 3rd Ed., p. 231. John Wiley, New York (1956).

[48] S. N. Koley, M. D. Kundu and A. N. Saha, Indian Oil Soap J. 30, 321 (1965), Chem. Abstr., 64, 14430 h (1966).

[49] K. A. Rao, Annamalai Univ. 6, 198 (1937); Chem. Abstr. 32, 8818^{7} (1938).

[50] B. J. Shreenivasan, Am. Oil Chem. Soc., 45, 259 (1968).

[51] A. K. Basu, Curr. Sci., 43, 410 (1974).

[52] P. Niogi and B. B. Adhicari, Prod. 7th Indian Soc. Cong, 1921; Chem. Abstr., 17, 3072^{6} (1933).

[53] N. V. Bringi, M. G. Kasbekar and K. Rabindran, Chem. Process, Eng., (India) 1, 151 (1967), 2, 25 (1968).

[54] N. V. Bringi and M. G. Kasbekar, Indian Patent 97, 196 (1968); Chem. Abstr., 82, 18918 h (1975).

[55] M. G. Kasbekar and N. V. Bringi, J. Am. Oil Chem. Soc., 46, 183 (1969).

[56] M. K. Kundu, and C. Bandopadhyay, J. Am. Oil, Chem. Soc., 46, 23 (1969).

[57] W. Horowitz, (Ed.) Official Methods of Analysis of the Association of Official Agricultural Chemists, 12th Ed., p. 481, Association of Official Agricultural Chemists, D. C. Washington (1955).

[58] M. K. Kundu, J. Chromatogr., 41, 276 (1969).

[59] M. K. Kundu, Fette Seifen Anstrichm., 72, 370 (1970).

[60] P. R. Ashurst, J. Sci. Res. Counc. Jam. 2, 4 (1971); Chem. Abstr., 75, 85128 u (1971).

[61] C. H. Hassall, K. Reyle and P. Feng, Nature 173, 356 (1954).

[62] W. A. Jacobs, J. Biol. Chem., 64, 379 (1925).

[63] P. C. Mahi, S. Roy and A. Roy, Experientta, 24, 1091 (1968).

[64] L. R. Row and C. Ruchmini, Indian J. Chem. 4, 36 (1966); Chem. Abstr., 64, 15948e (1966).

[65] O.C. Dermer and L.T. Crews, J. Am. Chem. Soc., 61, 2697 (1939).

[66] R.H. Cheney, Econ. Bot. I, 243 (1947).

[67] T. P. Hilditch and W. I. Stainsby, J. Soc. Chem. Ind., 53, 197 T (1934).

[68] C. Ratamapongse and A. J. Snowber, J. Sci. Food, Agric., 20, 137 (1969).

[69] A. Hartzell, Contribs. Boyce Thompson Inst. 15, 21 (1947); Chem. Abstr., 42, 4301 F (1948).

[70] E. Cheel and A. R. Penfold, J. Soc. Chem. Ind., 38, 74 T (1919).

[71] R. Kleiman, F. R. Earle and C. A. Wolff, Lipids, 4, 317 (1969).

[72] R. Kaleiman, Personal communication.

[73] M. Covello, Ann. Chim (Rome) 41, 780 (1951); Chem. Abstr., 46, 6409 h (1952).

[74] L. J. Haynes, J. R. Plimmer and W. M. Sue-Ho, J. Chem. Soc., 744 (1963).

[75] D. S. Seigler and W. Kawahara, Submitted to Biochem. Syst. Ecol.

[76] M. J. Chisholm and C. Y. Hopkins, Can. J. Chem., 36, 1537 (1958).

[77] L. Y. Hopkins and R. Swingle, Lipids 2, 258 (1967).

[78] K. L. Mikolajczak, C. R. Smith and L. W. Tjarks, Jr. Lipids 5, 812 (1920).

[79] K. L. Mikolajckak, D. S. Seigler, C. R. Smith and R. B. Bates, Jr. Lipids, 4, 617 (1969).

[80] C. Rimington, J. Oderstepoort Vet. Sci., 5, 445 (1935); Chem. Abstr., 30, 417 (1986).

[81] D. G. Steyn, and C. Rimington, J. Oderstepert Vet. Sci., 4, 51 (1935); Chem. Abstr., 30 (1935).

[82] C. S. Butterfield, E. E. Conn. and D. S. Seigler, Phytochemistry, 14, 993 (1975).

[83] W. Hubel and A. Nahrstedt, Phytochemistry, 14, 2723 (1975).

[84] D. S. Seigler, Phytochemistry, 13, 841 (1974).

[85] D. S. Letham, Phytochemistry, 5, 269 (1966).

[86] E. A. Braude and E. A. Evans, J. Chem. Soc., 3238 (1956).

[87] C. S. Marnil and N. O. Brace, J. Am. Chem. Soc., 70, 1775 (1948).

[88] C. C. Price, G. A. Chapher and T. V. Krishnamuriti, J. Am. Chem. Soc., 74, 2987 (1952).

[89] M. G. Kasbeker, R. R. Taleker and N. V. Bringi, Indian J. Chem., 10, 244 (1972).

[90] A. Vatakescherry J. Am. on Chem. Soc., 47, 295 A paper no. 521 (1970).

[91] D. S. Seigler Preparing for Biochem. Syst. Ecol.

[92] D. Charles, Q. G. Oli and S. M. Osman, Submitted to Chem., Ind.

[93] D. S. Seigler and C. S. Butterfield, Phytochemistry, 15, 842 (1976).

[94] D. S. Seigler, Personal Communication.

[95] D. S. Seigler, Chem., in Britain, 10, 339 (1974).

[96] D. S. Seigler and P. W. Price, Am. Nature, 119, 101 (1976).

[97] F. Feigl, Spot Tests, Vol. 1, 4th Ed., p. 263, Elsevier, New York (1954).

[98] T. J. Wood, Sci. Food Agric., 16, 300 (1965).

[99] F. Feigl, Spot Tests, Vol. 1, 4th Ed., p. 258. Elsevier, New York (1954).

[100] M. K. Kundu, J. Chromatogr., 46, 324 (1970).

[101] G. C. Guilbauli and D. N. Kramer, Anal, Chem., 38, 834 (1966).

[102] P. E. Kriedeman, Analyst, 89, 145 (1964).

[103] N. D. Mitchell, Can. J. Genet. Cytol., 16, 895 (1974); Chem. Abstr., 83. 39683 h (1975).

[104] W. O. Winkler, J. Assoc. Offic. Agric. Chem., 41, 282 (1958).

[105] L. J. Bellamy, the Infrared Spectra of Complex Molecules, p. 225 John Wiley, New York (1956).

[106] K. L. Mikolajczak, Unpublished Results.

[107] C. Litchfield, R. D. Harlow and R. Reiser, J. Am. Oil Chem. Soc., 42, 849 (1965).

[108] L. M. Soffer and M. J. Katz, Am. Chem. Soc., 78, 1705 (1956).

[109] A. O. Pittet, P. Pittersbacher and R. Muralidhara, J. Agric. Food, Chem., 18, 929 (1970).

[110] H. Sulzer, J. Depizzol and W. Buchi, J. Food Sci., 32, 611 (1967).

[111] L. J. Lascaray, Am. Oil Chem. Soc., 29, 362 (1952).

[112] F. A. L. Anet, J. Am. Chem. Soc., 84, 747 (1952).

[113] A. A. Bothner-By, and C. Naar-Colin, J. Am. Chem. Soc., 84, 743 (1962).

[114] R. U. Lemifux and E. Von Rudloff, Can. J. Chem., 33, 1710 (1955).

[115] E. Z. Eegriwe, Anal. Chem. 110, 22 (1937); Chem. Abstr., 31, 8442[3] (1937).

[116] H. C. Brown, Hydroboration, p-253. Benzamine New York (1962).

[117] C. Y. Hopkins, D. F. Ewing and M. Chisholm, J. Phytochemistry, 7, 619 (1968).

[118] B. Capon and D. Thacker, Proc. Chem. Soc., 369 (1964).

[119] T. K. Miwa, K. L. Mikolajczak, F. R. Earle and I. A. Wolff, Anal. Chem., 32, 1739 (1960).

[120] Official Methods of Analysis of the Association of Official Agricultural Chemists, 9th ed., 293 (1960).

[121] H. R. Bentley, K. G. Cunnigham and F. S. Spring, J. Chem. Soc., 2301 (1951).

[122] G. H. Shunk, B. O. Linn, J. W. Huff, J. L. Gilfillan, H. R. Skeggs and K. folkers, J. Am. Chem. Soc., 79, 3294 (1957).

[123] G. H. Butler and B. G. Butler, Nature, 187, 780 (1960).

[124] G. W. Butler, E. E. Conn., Biol. Chem., 239, 1674 (1964).

Publication/Conference Attended

[1] **"Cyanogenetic lipids from sapindaceous seed oils"** 2nd International Conference on Recent Advances in Biomedical and Therapeutic Sciences at B. U. Jhansi (6th-8th Jan., 2005)

[2] International Conference on Modern Trends in Forensic Science at B. U. Jhansi (13th-15th Feb., 2005)

[3] 13th National Symposium on Ultrasonics at B. U. Jhansi (21st-23rd Dec., 2004)